COMMUNAUTÉ FRANÇAISE DE BELGIQUE
UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

Assessing microbial diversity changes associated with different tillage and crop residue managements: study case in a loamy soil

Florine DEGRUNE

Essai présenté en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

Promoteur: Micheline VANDENBOL

2017

COMMUNAUTÉ FRANÇAISE DE BELGIQUE
UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

Assessing microbial diversity changes associated with different tillage and crop residue managements: study case in a loamy soil

Florine DEGRUNE

Essai présenté en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

Promoteur: Micheline VANDENBOL

2017

COMMUNAUTÉ FRANÇAISE DE BELGIQUE
UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

Assessing microbial diversity changes associated with different tillage and crop residue managements: study case in a loamy soil

Florine DEGRUNE

Essai présenté en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

Promoteur: Micheline VANDENBOL

2017

« Everybody is a genius. But if you judge a fish by its ability to climb a tree it will live its whole life believing that it is stupid»

Albert Einstein

Contents

Contents

Abstract

The major challenge of modern agriculture is to produce enough food for the growing population, and at the same time, minimize environmental harm. To meet this challenge, *Agroecology* aims to replace non-renewable external inputs with ecological processes to diversify the ecosystem services and attenuate the dis-services of agriculture. In this light, the ability to manage the soil microbiota, that has great effects on soil quality, is receiving attention. Plowing, the most widely used tillage practice in intensive agriculture has proven its efficiency in maximizing crop productivity, but its long term detrimental effects on soil quality, such as soil erosion and organic matter loss, have called for alternative tillage practices. However, the success of the implementation of these practices in Europe is still debated. In the upper part of Wallonia (Belgium), the soil is highly fertile and 80% of land is occupied mostly by intensive cropping systems. To date in Walloon cropping systems, few studies have explored the soil microbiota in association with different soil managements.

Here, we used a meta-barcoding approach to explore differences in soil microbial community structure under two contrasting tillage regimes, conventional (CT) and reduced tillage (RT), either with or without crop residue retention. The effects of these soil treatments were explored at different depths and during the growing season of two crops.

Our work demonstrated clear differences in microbial diversity between tillage regimes, but no clear differences between residue management practices. The observed differences appeared to be associated with differences in physical (e.g. structure and moisture) and chemical (nutrients) soil properties. Notably, the nutrient concentrations and moisture were higher under CT than under RT. Overall, soil under CT had higher or similar microbial diversity than under RT. Analysis of β-diversity revealed differences in the taxonomic structure of microbial communities. Certain microbial groups were more abundant under CT than under RT and *vice versa*. For example, mycorrhizal fungi, economically and ecologically important in agroecosystems, were more abundant under RT. Finally, the magnitude of tillage effects on the microbial diversity varied strongly with the sampling depth, whereas it varied moderately with the growing season.

This work highlighted CT was not necessarily unfavourable in maintaining microbial diversity when compared to RT. However, the study raises new questions regarding the impacts of microbial diversity changes on soil functioning. We encourage researchers to undertake further investigations into the functional role of microbiota in order to improve our understanding of agroecosystem functioning and its sustainability.

Résumé

Un défi majeur de l'agriculture moderne est de produire suffisamment de nourriture pour une population grandissante tout en garantissant l'intégrité de l'environnement. Afin de répondre à ce défi, l'*Agroécologie* a pour objectif de remplacer l'utilisation d'intrants non-renouvelables par des processus écologiques, afin de diversifier et de maximiser la production de services écosystémiques, et d'atténuer ainsi les dis-services liés à l'agriculture. Dans ce contexte, la capacité de manipuler le microbiote du sol, ayant des effets conséquents sur la qualité du sol, reçoit une attention toute particulière. La technique du labour, le type de travail du sol le plus largement utilisé en agriculture intensive, a d'ores et déjà prouvé son efficacité dans la maximisation de la production agricole. Mais, ces effets délétères sur la qualité du sol, observables sur le long terme, incluant notamment l'érosion des sols et des pertes en matières organiques, a suscité le développement de techniques alternatives. Cependant, force est de constater que le succès de l'implémentation de telles pratiques en Europe est encore fortement débattu. Dans la partie nord de la Wallonie (Belgique), le sol est très fertile et 80% des terres sont occupées par des grandes cultures gérées, pour la grande majorité, de manière intensive. Aujourd'hui, dans les systèmes wallons de grandes cultures, très peu d'études ont été lancées dans l'exploration du microbiote du sol en association avec différents types de gestions du sol.

Dans la présente étude, nous avons utilisé une approche de « meta-barcoding » pour explorer les différences de structure des communautés microbiennes du sol sous différents types de travaux du sol, conventionnel (CT) et réduits (RT), soit avec (R+) ou sans (R-) restitution des résidus de cultures. Ces effets ont également été explorés à différentes profondeurs du sol et au cours de la croissance végétale de deux cultures.

Notre étude démontre des différences significatives de diversité microbienne entre les types de travaux du sol, mais aucune différence claire n'est établie en fonction de la gestion des résidus de culture. Les effets observés ont été mis en relation avec des variations dans les propriétés physiques (ex : structure et humidité) et chimiques (nutriments) du sol. Notamment, la concentration en nutriments et l'humidité est plus élevées sous CT par rapport à RT. De manière générale, la diversité microbienne est plus élevée ou similaire sous CT par rapport à RT. L'analyse de la diversité β a révélé des différences majeures dans la structure taxonomique des communautés microbiennes du sol. Certains groupes microbiens sont plus abondants sous CT par rapport à RT et *vice versa*. Par exemple, les champignons mycorrhizien, économiquement et écologiquement importants dans les systèmes agricoles, sont plus abondants sous RT. Enfin, l'effet

Résumé

du travail du sol sur les communautés varie fortement fonction de la profondeur d'échantillonnage, alors qu'il varie plus modérément en fonction de la saison culturale.

Notre travail a permis de mettre en évidence que le labour conventionnel ne représentait pas nécessairement un frein dans le maintien de la diversité microbienne du sol par rapport au travail du sol simplifié. Cependant, notre étude soulève de nouvelles questions concernant l'effet de la diversité microbienne sur le fonctionnement global du sol. Nous encourageons de futures recherches à explorer le rôle fonctionnel du microbiote du sol afin d'améliorer notre compréhension du fonctionnement des agroécosystèmes ainsi que leur durabilité.

Acknowledgements

I wish to express my sincere gratitude to my thesis committee: Prof. Micheline Vandenbol, Prof. Bernard Bodson, Prof. Gilles Colinet, Prof. Aurore Degré, Prof. Marc Dufrêne, Dr. Christian Roisin (CRAw), and Dr. Pierre-Alain Maron (UMR Agroécologie, INRA Dijon, France), for insightful comments and encouragement, and also for the hard questions which prompted me to broaden my research to encompass various perspectives. I also wish to thank my supervisor Prof. Micheline Vandenbol for continuously supporting my PhD study and related research, for her motivation, and for her trust in my ability to conduct my research properly in a new field of microbial ecology.

A large part of my work would not have been possible without efficient collaboration with Dr. Martin Hartmann during my seven-month stay at WSL - the Swiss Federal Institute for Forest, Snow and Landscape Research in Zürich. His valuable professional qualities helped me to develop new expertise in bioinformatics and statistics, and his encouragement and patience substantially increased my confidence in my work. I would also like to express my gratitude to Beat Frey and Ivano Brunner for their warm-hearted welcome to their research unit at WSL. Besides my work at WSL, I also joined an enthusiastic research team and I wish to thank sincerely the different people with whom I spent some very nice moments: Aline, Thomas, and Virgine, the “French connection”, with whom I shared a memorable Swiss fondue and skiing time, Claude, the Swiss German introduced me to Swiss culture, and the other very nice people I met during my stay.

It is well known the best way to express feelings accurately is to use one’s mother tongue. Therefore I will continue in French to express my gratitude to my colleagues, friends, and family. Mon doctorat aura été une merveilleuse aventure dans laquelle je me suis épanouie et découverte notamment grâce aux nombreuses rencontres réalisées à Gembloux. Je voudrais commencer par remercier chaleureusement les collègues du laboratoire : Marjolaine, Nicolas, Sébastien, Michèle et Renée. Leur présence dans les moments plus difficiles de ma thèse ont été d’un soutien plus que réconfortant et un vrai moteur pour rester à flot. Au-delà de ces moments, nous avons vécu ensemble des vrais moments de partage et de dialogue.

Je voudrais ensuite remercier plus que chaleureusement le prof. Marc Dufrêne. Dès le début, il m’a transmis sa passion pour les grands jeux de données et les analyses multivariées. Au-delà de l’aspect purement technique, son soutien et sa confiance en mes capacités m’ont souvent donné des ailes pour aller de l’avant et persévérer.

Acknowledgements

Je voudrais remercier la plateforme AgricultureIsLife qui m'a permis de mener ma recherche à bien, ainsi que Sarah Garré et tous les doctorants qui en font partie, et plus particulièrement l'axe 2 : Marie, Marie-Pierre, Sophie et Nargish. Je remercie également toutes les personnes que j'ai croisées au cours de ma thèse que ça soit au détour d'un champ de blé pour des prélèvements de sol (Jean-Charles), ou au cours d'une réunion avec des échanges très stimulants et constructifs (Jean-Thomas et Fanny), ou encore autour d'un café.

Les amis ont également été un pilier important dans ma réussite : ma poulette préférée, Sandrine pour boire un petit porto et se raconter les derniers potins, ainsi que les potes de l'escalade qui n'ont jamais trop rien compris à mon travail, mais qui m'ont permis de « décrocher » pendant quelques heures salutaires par semaine : Bruno, Greg, Arnaud, Laurence, Thibaut, Mathieu, Kevin, Max, Vincent et tous les autres gros muscles (et moins gros muscles) de la salle à *Bebloc*.

Enfin, je remercie plus que chaleureusement mes amis très proches : Léo et Mimi, ainsi que ma famille : Phil et Liva, mes beaux-parents Michel et Francine, et enfin Gil, l'homme qui partage ma vie et qui a été d'un soutien plus que nécessaire dans cette aventure. Ils ont toujours été là pour moi et m'ont toujours soutenue autant dans ma vie personnelle que professionnelle.

List of figures

List of figures

List of tables

List of supplementary materials

List of abbreviations

AMF: arbuscular mycorrhizal fungi

bp: base pair

C: carbon

Ca: calcium

CT: conventional tillage

DNA: deoxyribonucleic acid

FAO: food and agriculture organization

HWC: hot water carbon

K: potassium

Mg: magnesium

N: nitrogen

Nmin: mineral nitrogen

NH4+: ammonium

NO3: nitrates

Na: sodium

OM: organic matter

OTU: operational taxonomic unit

P: phosphorus

R-: crop residue removal

R+: crop residue retention

rRNA: ribosomal ribonucleic acid

RT: reduced tillage

S: sulphur

Scientific publications and communications

PUBLICATIONS

FIRST AUTHOR

- **Florine Degrune**, Marc Dufrêne, Gilles Colinet, Sébastien Massart, Bernard Taminiau, Bernard Bodson, Marie-Pierre Hiel, Carine Nezer, Georges Daube, Micheline Vandenbol, **2015**. A novel sub-phylum method discriminates better the impact of management on soil microbial community. *Agronomy for Sustainable Development*, 35 (3): 1157-1166.
- **Florine Degrune***, Nicolas Theodorakopoulos*, Marc Dufrêne, Gilles Colinet, Bernard Bodson, Marie-Pierre Hiel, Bernard Taminiau, Carine Nezer, Georges Daube, Micheline Vandenbol, **2016**. No favorable effect of reduced tillage on microbial community diversity in a silty loam soil (Belgium). *Agriculture, Ecosystems and Environment*, 224: 12-21.
- Aboulkacem Lemtiri*, **Florine Degrune***, Sophie Barbieux, Marie-Pierre Hiel, Marie Chélin, Nargish Parvin, Micheline Vandenbol, Frédéric Francis, Gilles Colinet, **2016**. Crop residue management in arable cropping systems under temperate climate. Part 1: Soil biological and chemical (phosphorus and nitrogen) properties. A review. *Biotechnologie, Agronomie, Société et Environnement*, 20 (s1).

IN PREPARATION

- **Florine Degrune**, Nicolas Theodorakopoulos, Gilles Colinet, Bernard Bodson, Marie-Pierre Hiel, Bernard Taminiau, Carine Nezer, Georges Daube, Micheline Vandenbol, Martin Hartmann, **2017**. Dynamics of microbial communities over the growing season in two contrasted tillage regimes. In preparation to be published in *Frontiers in Microbiology*.

CO-AUTHOR

- Nicolas Theodorakopoulos, Margaux Lognoul, **Florine Degrune**, François Broux, Donat Regaert, Céline Muys, Bernard Heinesch, Bernard Bodson, Marc Aubinet, Micheline Vandenbol, **2016**. Increased expression of bacterial amoA during an N2O emission peak in an agricultural field. *Agronomy for Sustainable Development*.
- Marie-Pierre Hiel, Marie Chélin, Nargish Parvin, Sophie Barbieux, **Florine Degrune**, Aboulkacem Lemtiri, Gilles Colinet, Aurore Degré, Barnard Bodson, Sarah Garré, **2016**. Crop residue management in arable cropping systems under temperate climate. Part 2: Soil physical properties and crop production. A review. *Biotechnologie, Agronomie, Société et Environnement*, 20 (s1).

Scientific publications and communications

ORAL COMMUNICATIONS

NATIONAL CONFERENCE

- « Effets de pratiques agricoles de conservation sur la diversité microbienne et la dynamique des matières organiques dans des contextes pédologiques contrastés », Journée d'étude sur les sols (JES), Louvain-la-Neuve (BE), 6/07/2016.

MEETING PRESENTATIONS

- « Agriculture et vie microbienne », Livre blanc, Gembloux ABT (BE), 26/2/2014.
- « Farming effects on soil microbial diversity under contrasting soil types », Agroscope de Zürich, (CH), 20/4/2016.

POSTER COMMUNICATIONS

INTERNATIONAL CONFERENCES

- « Impact of agricultural practices on soil microbial communities in Belgium », 2nd Thünen symposium on soil metagenomic, Braunschweig, **Germany**, 11-13/12/2013.
- « Detecting microbial patterns in relation to soil agricultural practices and the plant development stage », The first Global Soil Biodiversity conference (GSB), **Dijon**, France, 2-5/12/2014.
- « Soil microbial community composition changes according to the tillage practice and plant development stage », European Geosciences Union, Vienne, **Austria**, 12-17/4/2015.
- « No favorable effect of reduced tillage on microbial communities in a silty loam soil (Belgium) », Ecology of soil microorganisms, Prague, **Czech Republic**, 29/11-3/12/2015.
- « Mise en place de pratiques agricoles de conservation : quel impact sur la vie du sol? », Traits Ecologiques et Biologiques des organismes du sol (TEBIS), **Toulouse**, France, 3-5/10/2016.

Chapter I. General Introduction

1. General context

Modern agriculture: benefits, trade-offs and future challenges

Since the Second World War, major advances in scientific and technological innovations have profoundly changed the face of modern agriculture. During this period, called the Green Revolution, the development of new high-yield crop varieties, the discovery of synthetic fertilizers, pesticides and herbicides, and technical improvements in mechanization have led to a considerable increase in global food production. The global production of cereal crops more than doubled between 1960 and 2000 (Tilman, 1999). With the main objective of the maximization of crop production, modern agriculture has become intensive.

Although the success of intensive agriculture in meeting the global food demand was immense, major environmental concerns have emerged (Tilman, 1998, 1999). For example, the systematic and excessive use of fertilizers causes the eutrophication of lakes and rivers (Smith et al., 1999), air pollution from increases in NOx emissions in the atmosphere (Smith et al., 1997) and the alteration of biodiversity (Allison et al., 2007; Vitousek et al., 1997). As a consequence, and alongside the ever increasing global population, which a United Nations report (2015) estimates will increase one-third by 2050, there is an urgent need to develop strategies to design new agricultural systems that are more productive, stable, and resilient while minimizing their environmental impact (Foley, 2005; Tilman, 1998).

Ecological intensification is a promising approach by which to meet the future challenges of agriculture (Bommarco et al., 2013; Doré et al., 2011). This concept is a part of *agroecology*, a larger concept that also includes socioeconomic aspects (Hatt et al., 2016), and it applies ecological principles to agricultural practices. The main objective of agroecology is to replace non-renewable external inputs with ecological processes (e.g. pollination, nutrient cycling, carbon decomposition) in order to diversify the ecosystem services (**Box 1**) and reduce the harm of agriculture (e.g. habitat loss, nutrient runoff) (Zhang et al., 2007).

Unlike intensive agriculture, agroecological approach does more than just promote the production service (e.g. crop production), it promotes the diversity of ecosystem services, agricultural-based as well as non-agricultural-based (**Figure 1**) (Foley, 2005). Agroecosystems that provide multiple

ecosystem services are expected to be more resistant and resilient to external perturbations such as global climate change (Soliveres et al., 2016).

To date, however, even if the concept of agroecology is well known in South America for more than a decade (Altieri, 1999), how to successfully implement it in the wide range of climatic and pedological conditions found in Europe is still questioning.

Box 1. Definitions of ecosystem services

As defined by the Millennium Ecosystem Assessment (2005), ecosystem services are the benefits humans obtain from ecosystems. They are grouped into four categories:

1. *Supporting services*, such as nutrient cycling and soil formation.
2. *Regulating services*, such as pest control, crop pollination, climate regulation, and water purification.
3. *Provisioning services*, such as food, fiber, fuel, and water.
4. *Cultural services*, such as education, recreation, and aesthetic value.

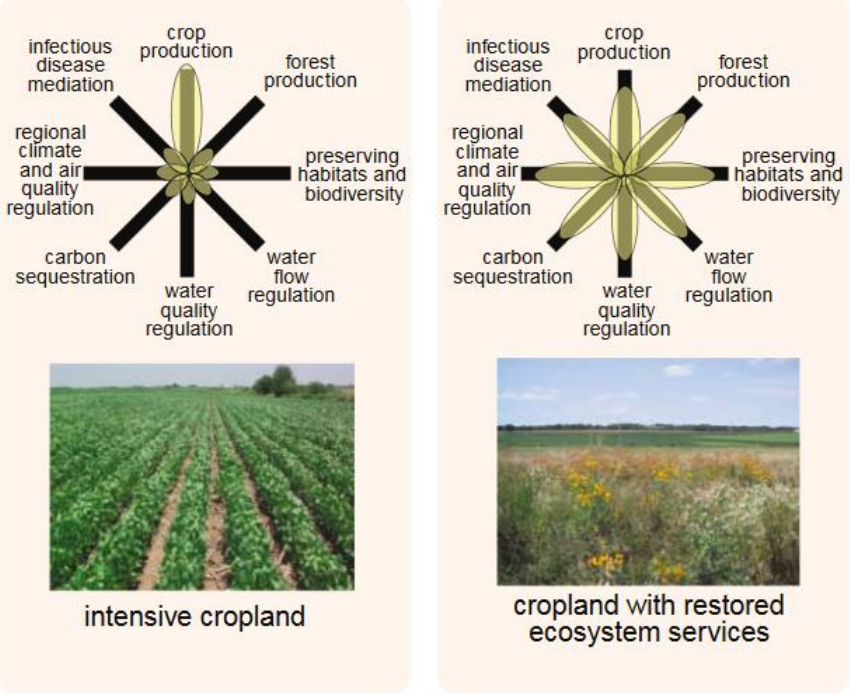


Figure 1 Conceptual framework for comparing trade-offs of ecosystem services in intensive (left) and ecological-based (right) farming (according to Foley, 2005).

Soil microbiome: a key factor for soil functioning

Soil biota maintain the soil functioning, and thus influence strongly agricultural productivity (Altieri, 1999; Barrios, 2007). Even if this productivity is driven by complex interactions between soil biota and abiotic factors (Kibblewhite et al., 2008), most soil processes related to organic matter transformation and nutrient cycling are mediated by microorganisms. Microorganisms are the most abundant and diverse group of soil organisms with one single gram of soil estimated to contain tens of thousands of species (Fierer et al., 2007b; Roesch et al., 2007). These species influence substantially the functioning of the soil ecosystem (Aislabie et al., 2013; East, 2013; Nannipieri et al., 2003; van der Heijden et al., 2008) and thus contribute to important agricultural services (e.g. food and fibre) and non-agricultural services (e.g. water quality and supply, erosion control, atmospheric composition and climate regulation) (Kibblewhite et al., 2008).

Microorganisms also contribute to soil aggregate formation and aeration, as well as carbon sequestration in agroecosystems (Six et al., 2006) that are important aspects in agricultural productivity and environmental issues. Some specific "key" groups such as the N-fixing bacterial symbionts of the legumes (Cleveland et al., 1999; Van Der Heijden et al., 2006), arbuscular mycorrhizal fungi (AMF) (Van der Heijden et al., 1998) and plant growth promoting rhizobacteria (PGPR) (Rodríguez and Fraga, 1999) can substantially enhance plant productivity by supplying growth-limiting nutrients. In addition, since soil microorganisms can respond rapidly to environmental changes, they can be used as indicators to evaluate soil quality (Schloter et al., 2003).

To date, there is evidence that a loss in microbial diversity can affect important soil processes such as the nitrogen cycling (Philippot et al., 2013b). As a result, understanding and managing the soil biodiversity so that key soil processes are optimized is a major challenge in the context of ecological intensification, i.e. developing strategies to develop ecological-based agriculture (Lemanceau et al., 2014).

However, given the large abundance and diversity of soil microbiota, exploring its structure is still a challenge. In the past, some methodological limitations have prevented the exploration of soil microbial communities. Until the 1990s, most investigators used laboratory culture-dependent methods to explore microorganisms. However, only 1%-10% of microorganisms can be cultured.

The culture-dependent methods suffer from low detection, resolution and throughput and therefore, these methods are quite limited in capturing the complexity of microbial structures.

With the recent development of DNA-based methods in the 1990s, which bypass the need for culturing, and with the rapid improvements in high-throughput DNA sequencing over the last decade (Cardenas and Tiedje, 2008; Glenn, 2011), we are now able to get insights into the immense taxonomic and functional structures of microbial communities in complex environments such as soils (Fierer et al., 2007b; Jung et al., 2016; Urich et al., 2008).

Soil tillage: benefits and detrimental effects

Soil tillage, which refers to all mechanical actions performed on soil, is an important component of agroecosystem management, and it has a significant impact on the soil properties (e.g. soil structure[1], nutrient availability, and soil biological activity). Among the wide range of contemporary soil tillage practices, plowing – conventional tillage – is the most ancient. This has been used for centuries to optimize seed germination and root development by modifying soil conditions (Titi, 2002). Plowing i.e. soil conversion combined with burying plant residues in a depth of between 15 and 40 cm (according to the type of the machinery employed) has many short-term benefits, including notably weed and pest control, temporary relief of soil compaction, and the incorporation of plant residues, fertilizers and pesticides. Plowing proved to be effective in intensive agriculture in maximising the crop production.

Besides these short-term benefits, however, there are long-term detrimental effects, such as soil erosion and loss of soil organic matter (Montgomery, 2007; Six et al., 1999). This has led to the development of alternative conservation tillage practices (e.g. reduced tillage). These soil practices aim to minimise soil disturbance, prevent soil degradation, and enhance both soil quality and crop productivity in agroecosystems (Hobbs et al., 2008; Pittelkow et al., 2014). To date, there is a wide range of conservation tillage practices available (**Figure 2**) that differ in terms of the volume of soil disturbed, the intensity and frequency of soil disturbance, and the amount of crop residues covering soil (Reicosky, 2015).

[1] Soil structure refers to the spatial arrangement of soil aggregates that determines the pore size and connectivity.

In Europe, however, the success of the implementation of such conservation practices is still unclear and debated. Success seems to depend on a variety of different factors, including crop rotation, soil type, and regional climate. In certain conditions, the implementation of conservation practices can lead to detrimental effects on soil quality, such as increased bulk density and acidity near surface (Basch et al., 2015).

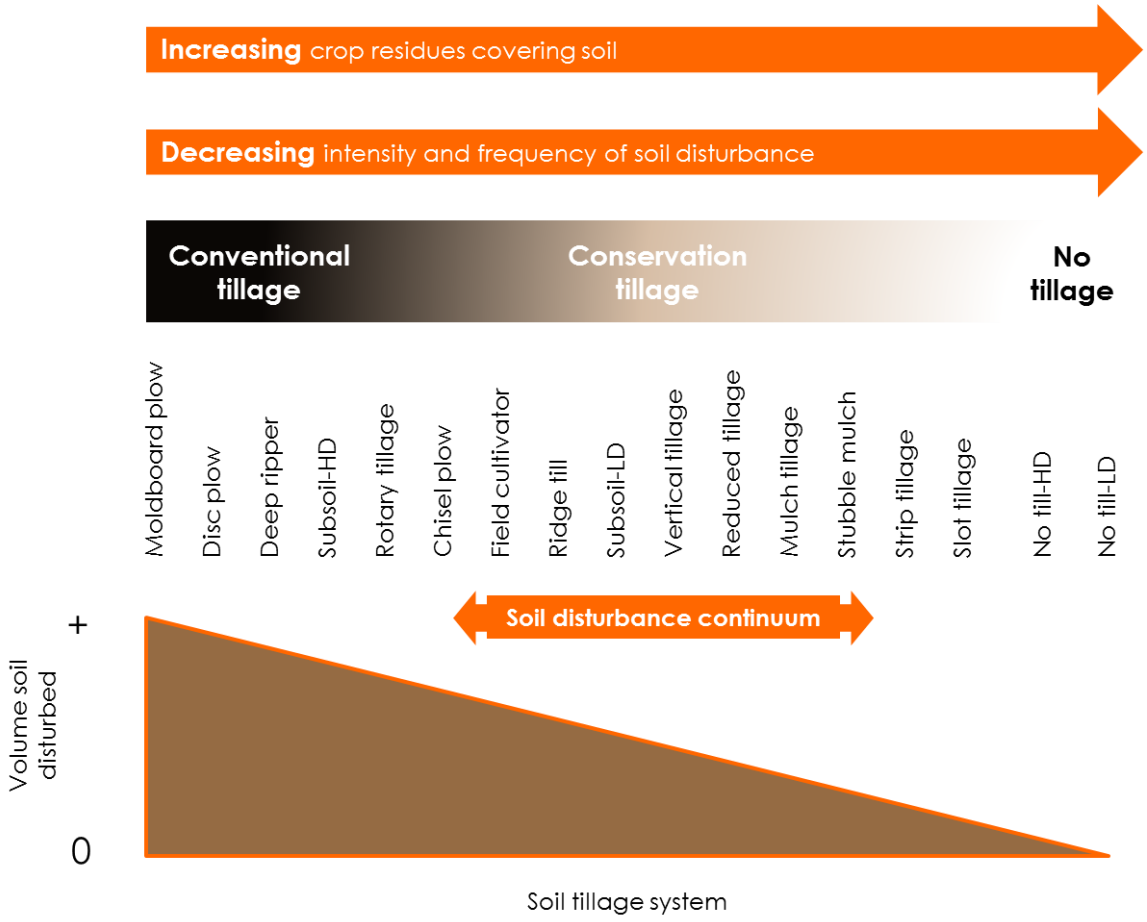


Figure 2 Schematic representation of soil disturbance continuum associated with different tillage practices ranging from conventional tillage (highest soil disturbance) to no tillage (lowest soil disturbance). HL=high disturbance and LD=low disturbance. Source: modified after Reicosky (2015).

2. Context, objectives and outline of the thesis

Context

The present study was part of the **AgricultureIsLife** research platform, a larger project launched in 2013 at Gembloux Agro-Bio Tech. The research platform is based on collaborative work associated with a multidisciplinary approach involving several scientists for the exploration of innovations to improve the sustainability of agriculture in temperate Western Europe (Monty et al., 2016).

Of the five key innovation themes of the research platform, one was dedicated to the *management of different quantities of crop residues under different tillage practices (conventional vs. conservation) and its impact on agronomical (e.g. crop yield, weed control) and soil (e.g. soil structure, microbial diversity) properties.*

This theme is of particular importance in the upper part of Wallonia as the region is mostly occupied by cropping systems (**Figure 3**), with 80% of land used for agriculture. As a result, questions related to the implementation of conservation soil practices and its impact on the structure of soil microbiota are strongly oriented to developing more sustainable agriculture.

To date, however, the way the structure of the soil microbiota responds to soil tillage practice (conventional or conservation) associated with different quantities of crop residues in our agricultural region remains unexplored.

Even if a few studies have examined the structure of the soil microbial communities in relation to different soil tillage practices associated with different crop residue managements while using high-throughput sequencing technology (Carbonetto et al., 2014; Dorr de Quadros et al., 2012; Jiménez-Bueno et al., 2016; Navarro-Noya et al., 2013a; Sengupta and Dick, 2015), most of them are located in an area of the world characterized by specific local edaphic and climatic conditions, and strongly at variance with conditions in upper Wallonia, which in turn can lead to contrasting results.

Therefore, given the strong dependence of the soil properties caused by soil tillage on local edaphic and climatic conditions, the impact of different soil management practices on the structure of microbial communities in upper Wallonia is of primary interest in the context of developing more productive, stable, and resilient agriculture while minimizing its environmental impact in the region.

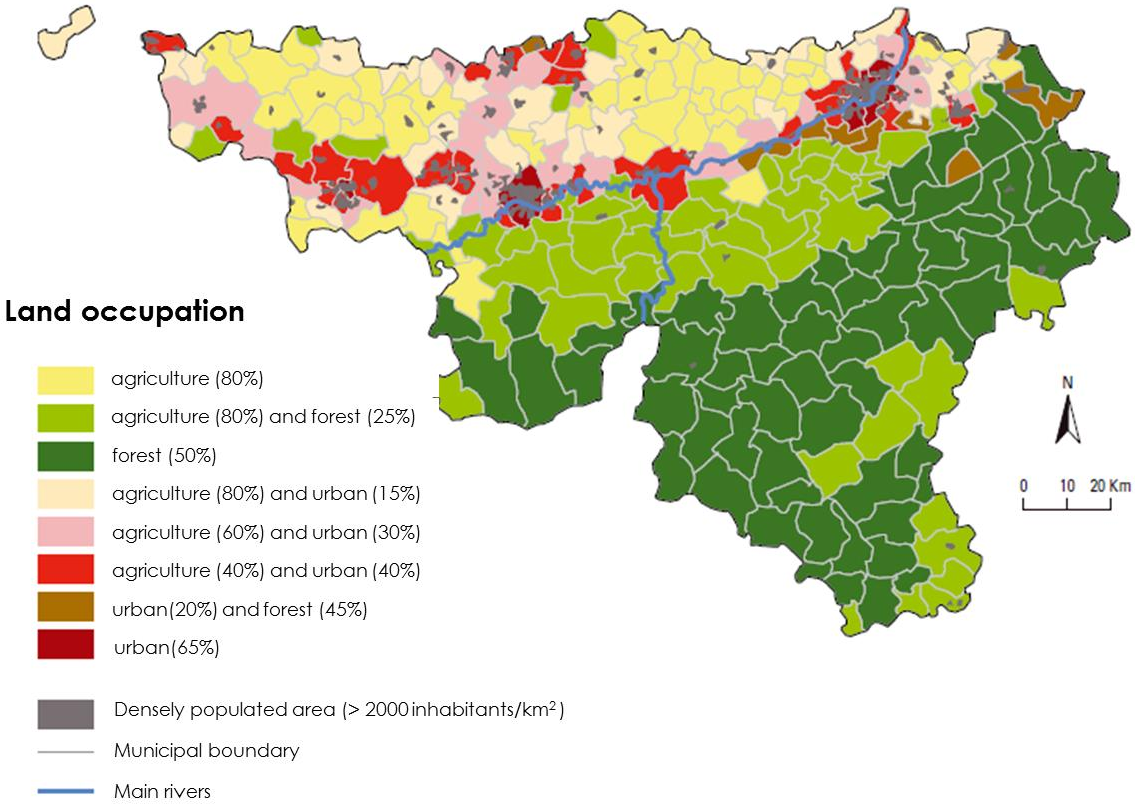


Figure 3 Map showing land use in Wallonia (South part of Belgium). The upper part of Wallonia (north of the river) is mostly occupied by cropping systems. *Source: SPF Economie - DGSIE (INS-Occupation du sol - 2004).*

Objectives

In our study, we aimed **(1)** to identify the effects of different soil treatments – tillage regime (CT: conventional and RT: reduced tillage) and crop residue management (R+: residue retention and R-: residue removal) – on the structure of soil microbial communities, i.e. on α- and β-diversity (Whittaker, 1972) (**Box 2**) and **(2)** to identify the soil-treatment-related soil physical and chemical parameters that might explain the observed effects. We further investigated soil treatment effects in relation to soil depth (top soil: 0 to 5 cm, deep soil: 15 to 20 cm) and over the growing season of two crops: *Vicia faba* (fababean) and *Triticum aestivum* (wheat). To achieve the goals, we aimed to answer the following questions:

Box 2: Diversity metrics used to infer the structure of microbial communities.

Alpha-diversity (*α*): it refers to the *species diversity* in a single sample unit, and was assessed using three metrics: the number of species (S), the Shannon index, and the Smith-Wilson evenness index (Smith and Wilson, 1996).

Beta-diversity (*β*): it refers to the *difference in species composition* among sampling units, and was assessed using the Bray-Curtis dissimilarity metric (Bray and Curtis, 1957), calculated from differences

1. **Do soil treatments affect microbial α- and β-diversity and soil physical and chemical parameters? Is there a relationship between microbial patterns and soil parameters?**
2. **Do depth and season modify the magnitude of soil treatment effects on microbial α- and β-diversity?**
3. **Can we relate differences in α- and β-diversity to ecological meanings?**

To answer these questions, we tested the following hypotheses (**Table 1**).

Table 1 Statement of our assumptions.

H1	In general, microbial α- and β- diversity change with the tillage regime, RT promoting higher species diversity than CT.
H2	Under CT, microbial α- and β- diversity are similar in the top soil and deep soil. Under RT, microbial α- and β- diversity change with depth so that species diversity is higher in the top soil than in the deep soil.
H3	The effect of tillage regime on microbial α- and β- diversity varies during the growing season of the plant considered (fababean or wheat), and the difference between RT and CT diminishes over time.

RT = reduced tillage; CT = conventional tillage; top soil = 0 to 5 cm; deep soil = 15 to 20 cm.

Outline

This study is a compilation of scientific papers that have been published or are being reviewed. It is structured as follows:

- **Chapter II** reviews the state of the art on the effects of crop residue management on biological, chemical, and physical properties in arable cropping systems under a temperate climate. This review is a collaborative work that has been integrated into the special issue 'AgricultureIsLife project'. Part 1 is presented in this chapter. Reference: *Lemtiri, Degrune et al. (2016), in Biotechnologie, Agronomie, Société et Environnement (BASE).*
- **Chapter III** (H1) describes an original method that improves the resolution of the analysis used to detect microbial patterns related to the tillage regime and crop residue management. To test the method, data collected in 2013 at the seedling stage of *Vicia faba* was used. Reference: *Degrune et al. (2015), in Agronomy for Sustainable Development (ASD).*
- **Chapter IV** (H2) explores the microbial patterns associated with tillage regime and crop residue management at different soil depths (0 to 5 cm and 15 to 20 cm) at the grain filling stage for *Triticum aestivum*. Reference: *Degrune et al. (2016), in Agriculture, Ecosystems & Environment.*
- **Chapter V** (H3) summarizes the data of the entire two-year experiment. It focuses on the effects of tillage regime and crop residue management both overall and in relation to the growing stage of *Vicia faba* or *Triticum aestivum*. Reference: *Degrune et al. (2017, in prep), in Frontiers in Microbiology.*

Finally, in **Chapter VI** we discuss the main results and we also include consideration of prospects and potential improvements.

3. Site description, experimental design and sampling protocol

Site description

The SOLRESIDUS long-term experiment is located on the experimental farm of Gembloux Agro-Bio Tech. The climate of the region is oceanic temperate and the soil type is classified as *Cutanic Luvisol* according to the FAO. The soil texture is silt loam and largely dominated by silt (70-80%), clay (18-22%) and sand (5-10%), and the organic matter is characterized by a C:N ratio between 10 and 12.

The chemical soil fertility of the experiment was evaluated at the beginning of the project, in 2011 (Colinet et al., 2013) for the following soil parameters: pH, total organic carbon and available elements (P, K, Ca, Mg). The summary of descriptive statistics is provided in **Table 2** and the spatial variability is depicted in **Figure 4** to **Figure 9**.

Table 2 Descriptive statistics of SOLRESIDUS soil parameters.

N[1]=107	**pHKCl**	**TOC[4]**	**P**	**K**	**Ca**	**Mg**
		$g.kg^{-1}$		$mg.100g^{-1}$		
Mean±sd[2]	6.79±0.19	12.7±1.2	14.9±4.9	16.2±2.7	256±37	8.3±1.7
CV[3] (%)	2.8	9.4	32.9	16.7	14.4	20.5
Min/Max	6.40/7.30	9.4/16.0	6.5/24.8	10.5/22.2	205/369	4.6/11.8

[1]number of samples/[2]standard deviation
[3]coefficient of variation/[4]total organic carbon

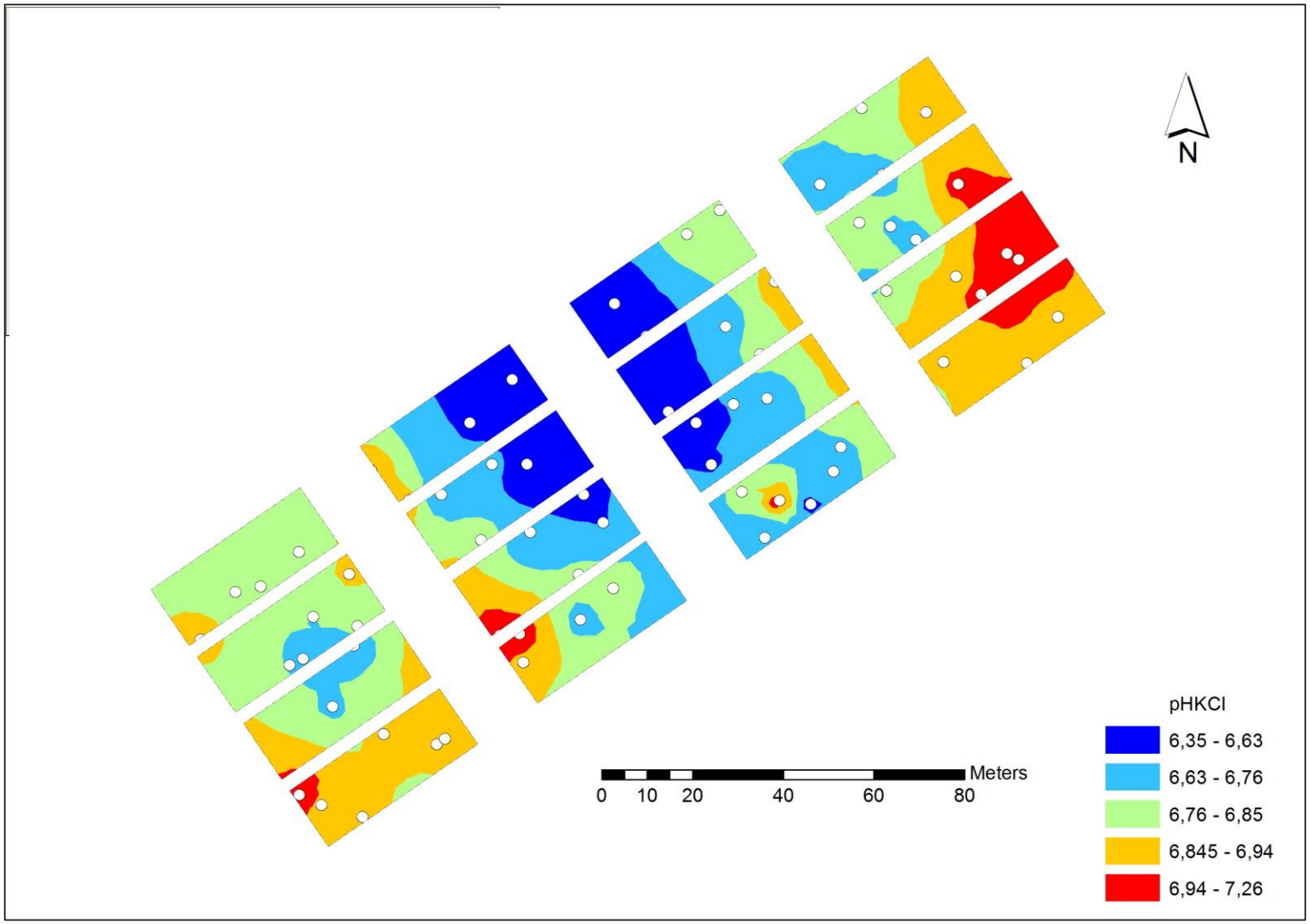


Figure 4 Map of soil pH in the 0-30 cm soil horizon in SOLRESIDUS experiment. The map was obtained using the Kriging method for interpolation with the software ArcGIS.

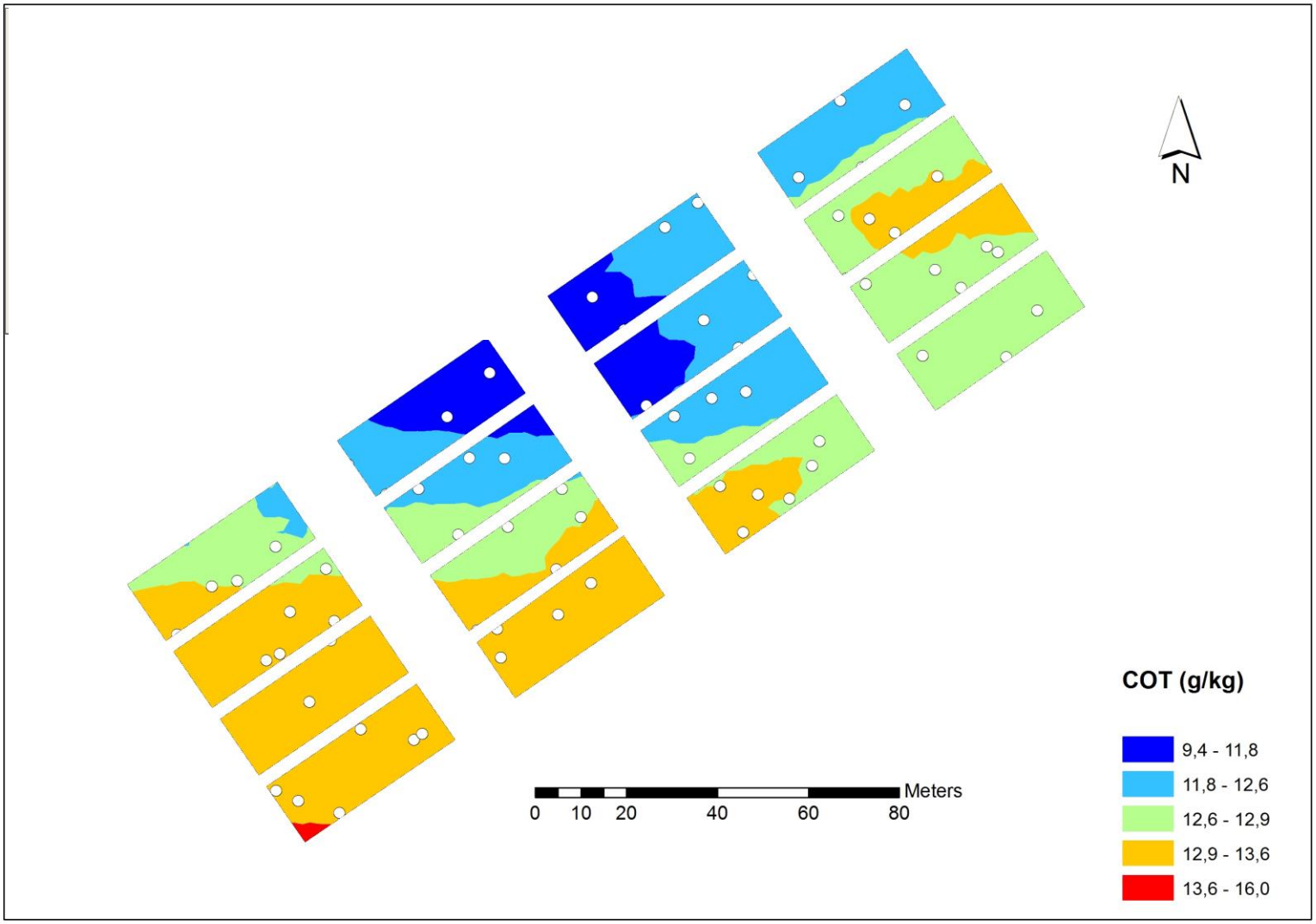


Figure 5 Map of soil total organic carbon (TOC) in the 0-30 cm soil horizon in SOLRESIDUS experiment. The map was obtained using Kriging method for interpolation with the software ArcGIS.

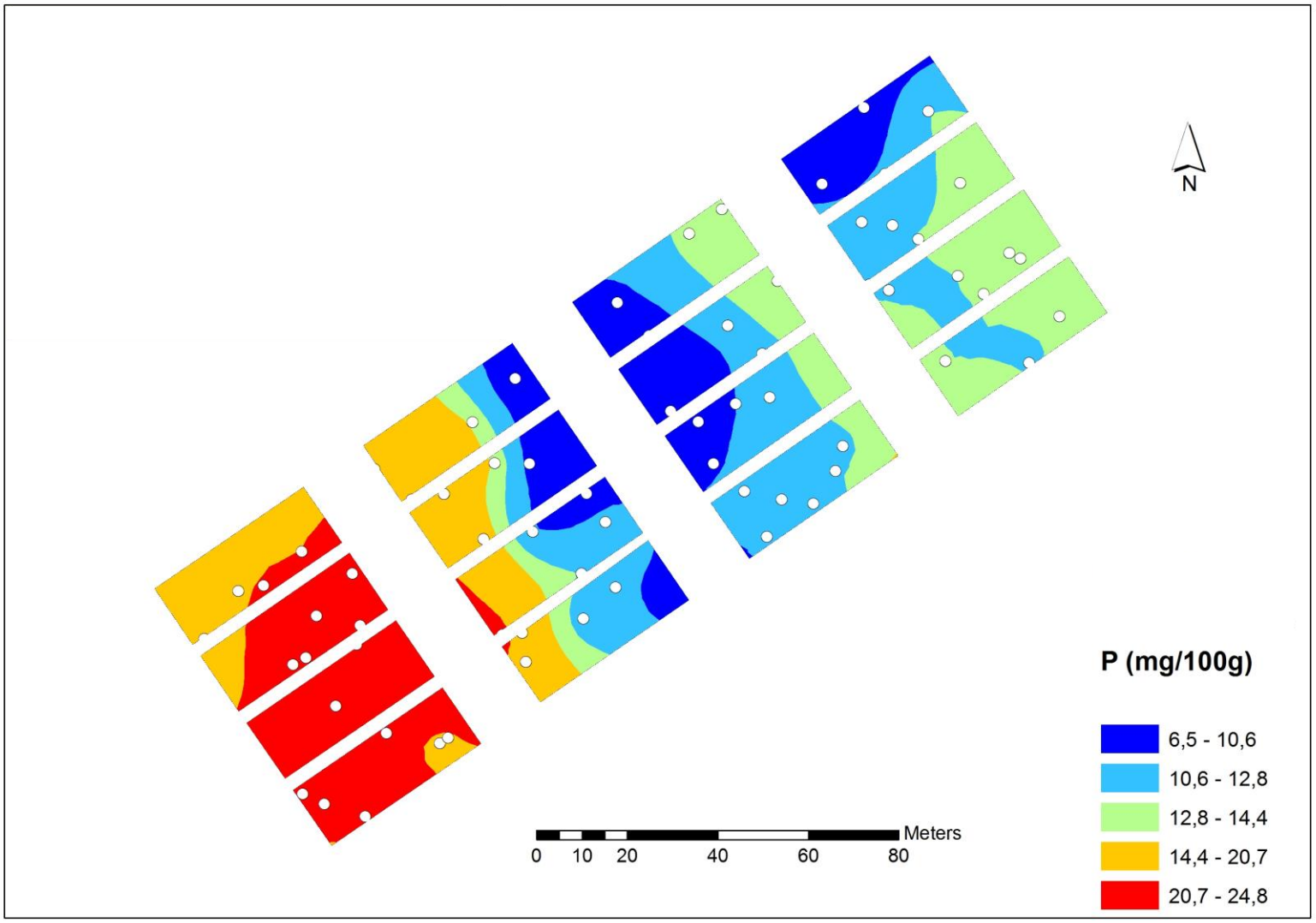


Figure 6 Map of phosphorus (P) availability in the 0-30 cm soil horizon in SOLRESIDUS experiment. The map was obtained using the Kriging method for interpolation with the software ArcGIS.

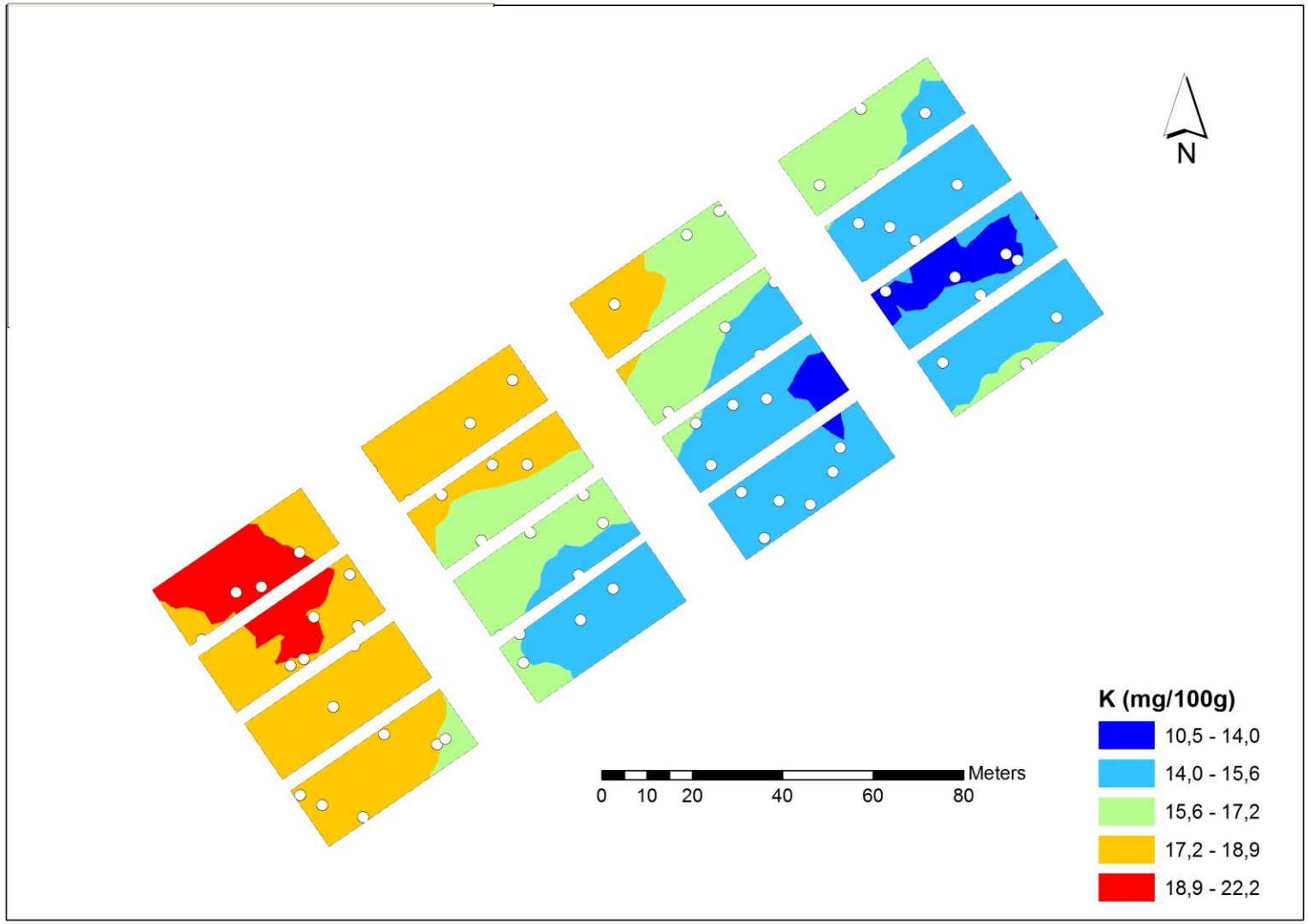


Figure 7 Map of the potassium (K) availability in the 0-30 cm soil horizon in SOLRESIDUS experiment. The map was obtained using the Kriging method for interpolation with the software ArcGIS.

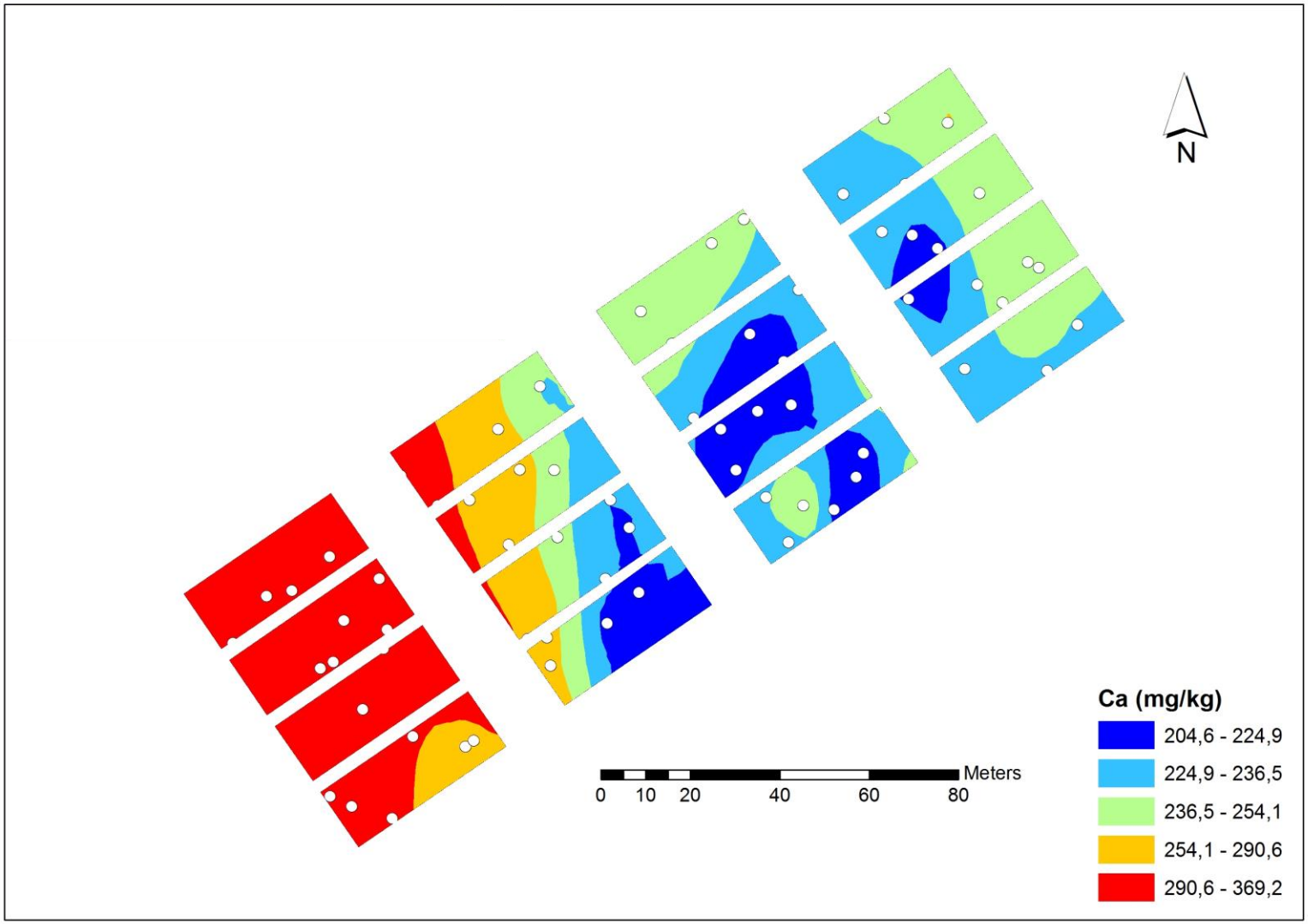


Figure 8 Map of potassium (K) availability in the 0-30 cm soil horizon in SOLRESIDUS experiment. The map was obtained using the Kriging method for interpolation with the software ArcGIS.

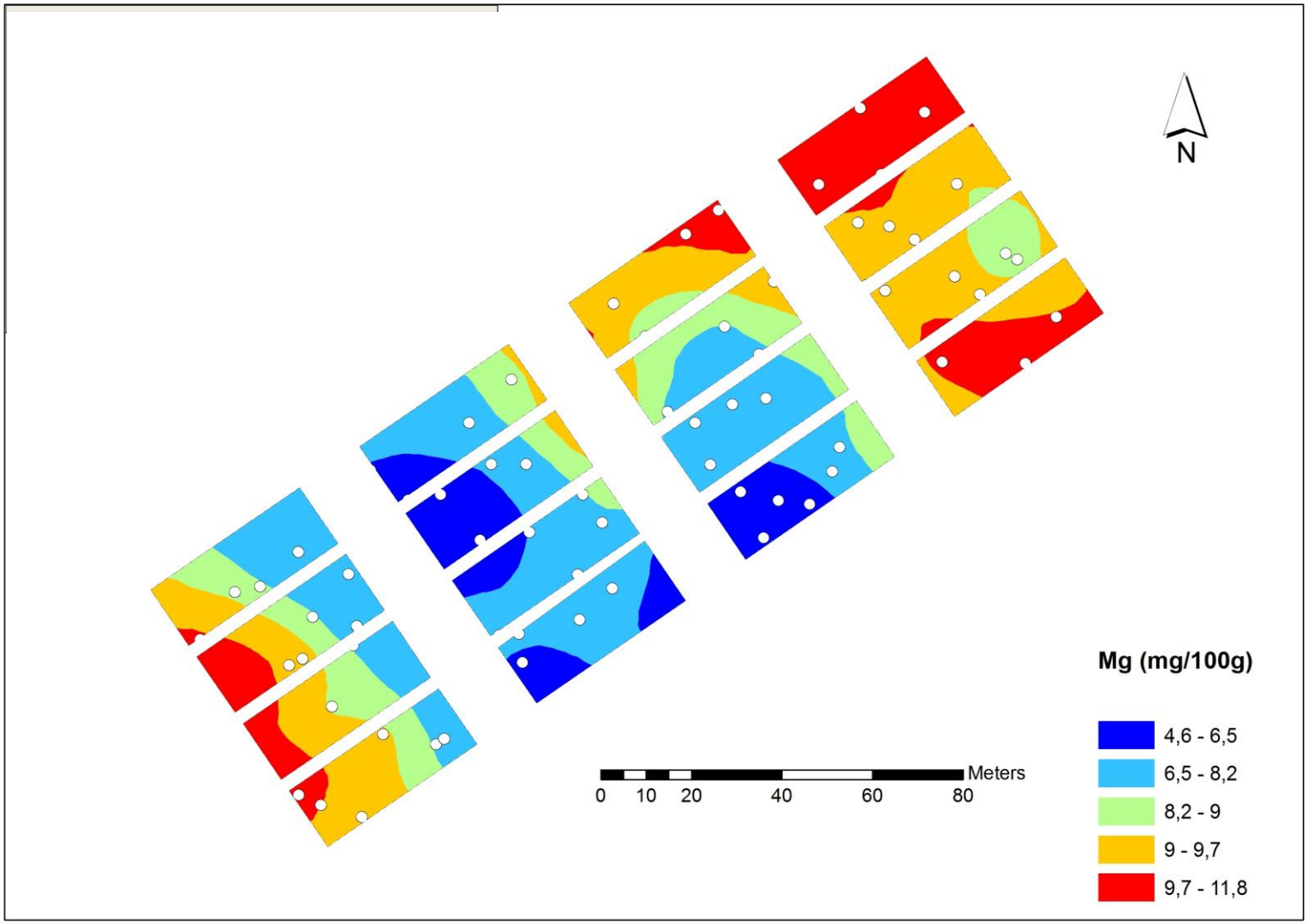


Figure 9 Map of magnesium (Mg) availability in the 0-30 cm soil horizon in SOLRESIDUS experiment. The map was obtained using the Kriging method for interpolation with the software ArcGIS.

Experimental design

The experimental design of SOLRESIDUS and the different soil treatments have been applied since autumn 2008. Before 2008, the site was under conventional tillage. The design of the experimental field consisted of a Latin square arrangement with 16 plots (**Figure 10**): four soil treatments replicated four times. Each plot is 40 meters long and 15 meters wide. The different soil treatments were as follows: conventional tillage with residue removal (CT/R-), conventional tillage with residue retention (CT/R+), reduced tillage with residue retention (RT/R+), and reduced tillage with residue removal (RT/R-).

The residues removed consisted of harvestable straw, while stubbles and chaffs were left on the field in both R+ and R-. In all plots, stubble breaking at a depth of 10 cm was performed to bury the residues. After stubble breaking, plowing to a depth of 25 cm was applied only to the CT plots, with a moldboard plow (**Figure 11**). Seedbed preparation was identical on all plots and was performed at a depth of 7 cm.

Fertilizer, fungicide, and weedkiller treatments were applied equally to each plot. The dates of the different soil operations are provided in supplementary material (**S1**).

Crops are rotated on the studied field and crop history is as follows: *Brassica napus* (2009), *Triticum aestivum* (2010, 2011 and 2012), *Vicia faba* (2013), and *Triticum aestivum* (2014).

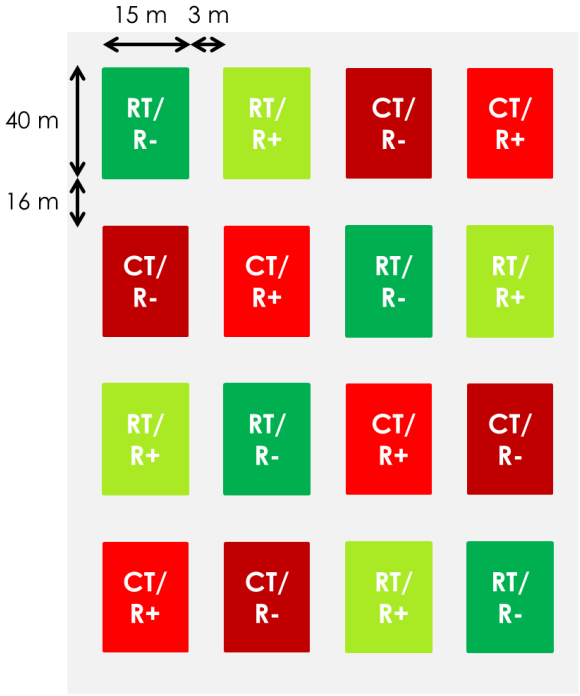


Figure 10 Experimental design of the SOLRESIDUS experiment. Each treatment, i.e. CT/R+, CT/R-, RT/R+ and RT/R- is replicated four times in a Latin square arrangement. *RT=reduced tillage*, *CT=conventional tillage*, *R+=crop residues retention*, and *R-=crop residues removal*

Figure 11 Moldboard plow employed on CT plots to mix and invert soil to a depth of 25 cm. Image credit: Marie-Pierre Hiel.

Sampling protocol

The sampling protocol is presented in **Figure 12**. Soil samples were collected from the deep soil (15 to 20 cm) of each of the 16 plots in 2013 (faba bean) and 2014 (wheat), at different growing stages (seedling, leaf development, and flowering stages for faba bean, tillering and grain filling stages for wheat). The soil sampling dates are provided in **S1**. An additional sampling of the top soil horizon (0 to 5 cm) was performed at the grain filling stage for wheat.

Each soil sample was a composite of six randomly selected soil cores of 5 cm length and 2 cm diameter, collected with an auger as close as possible from the stem and in a delimited area of $6m^2$ sizes moving every year. One single composite soil sample was collected on each plot.The collected soil samples were stored at 4°C for downstream chemical and physical analysis, and at -20°C for downstream microbiological analysis.

In our study, the choice of the sampling depth was based on two main criteria: (1) low dependency of soil conditions on atmospheric fluctuation – we expected more fluctuations at the top soil horizon, and (2) a maximisation of differences in physical and chemical soil conditions between CT and RT – the deep soil horizon in RT was undisturbed for the last 6 years, while the top soil horizon was disturbed through stubble breaking. The soil below 20 cm was not considered as the plow-pan occurred around that depth.

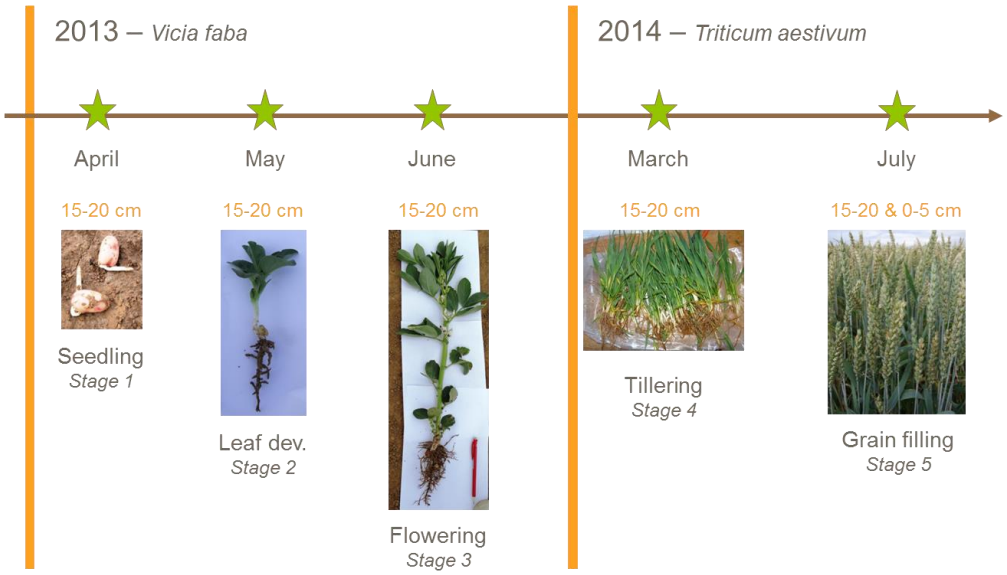


Figure 12 Schematic representation of the soil sampling protocol performed on all plots of the SOLRESIDUS experiment. Each sampling is represented by a star and the soil depth of the sampling is in orange text.

On each soil sample (**Figure 13**), we determined (1) bacterial and fungal α- and β- diversity using a molecular approach and (2) the soil physical and chemical properties including the water-extractable elements (P, Mg, Na, K, Ca, HWC), the content of nitrates (NO3), ammonium (NH4) and mineral nitrogen (Nmin), the pHKCl, and the water content. The methods used to determine the Soil physical and chemical properties are detailed in chapters III to V. The methodology used to characterize the microbial diversity is explained in a nutshell in the next section.

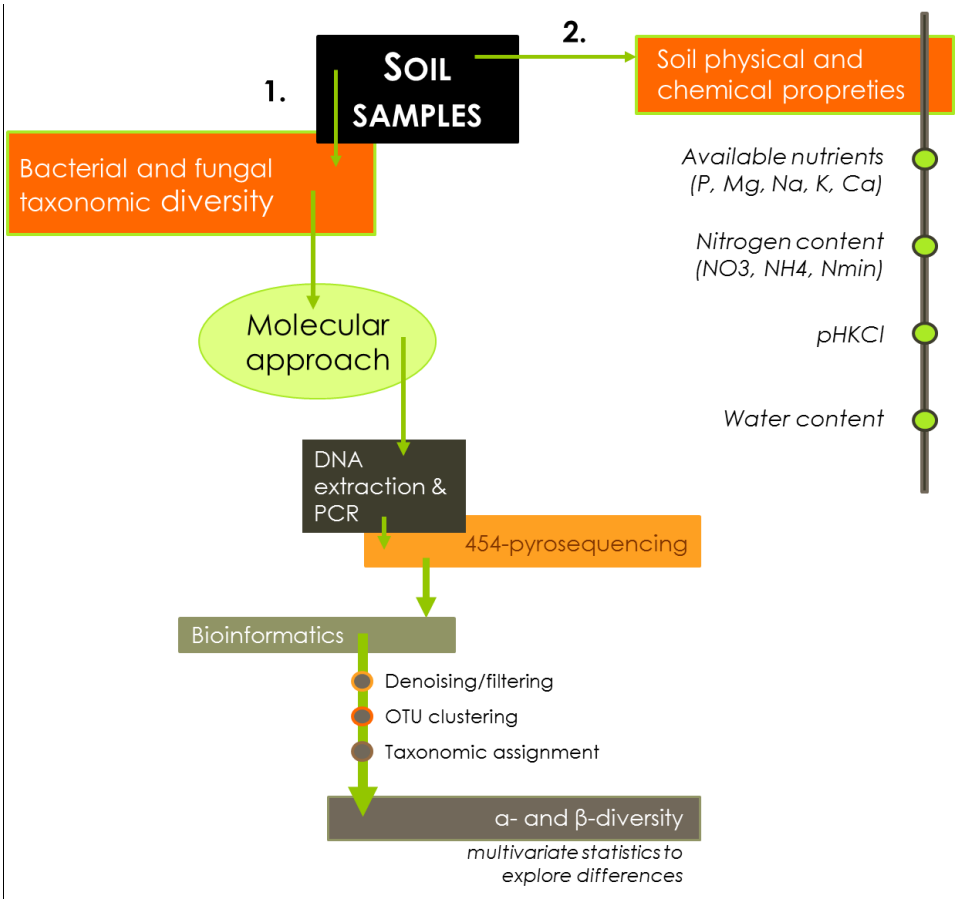


Figure 13 Diagram of what variables are studied. On each soil sample, (1) bacterial and fungal diversity was determined using molecular approach and (2) the soil physical and chemical properties were evaluated. The different steps to evaluate the taxonomic diversity of microbial communities is detailed and includes the DNA extraction, amplification of genetic marker, bioinformatics to cure and assign the sequences, and statistical analysis to explore the differences in taxonomic diversity among the different soil treatments.

5. Methods used to explore the soil microbiome

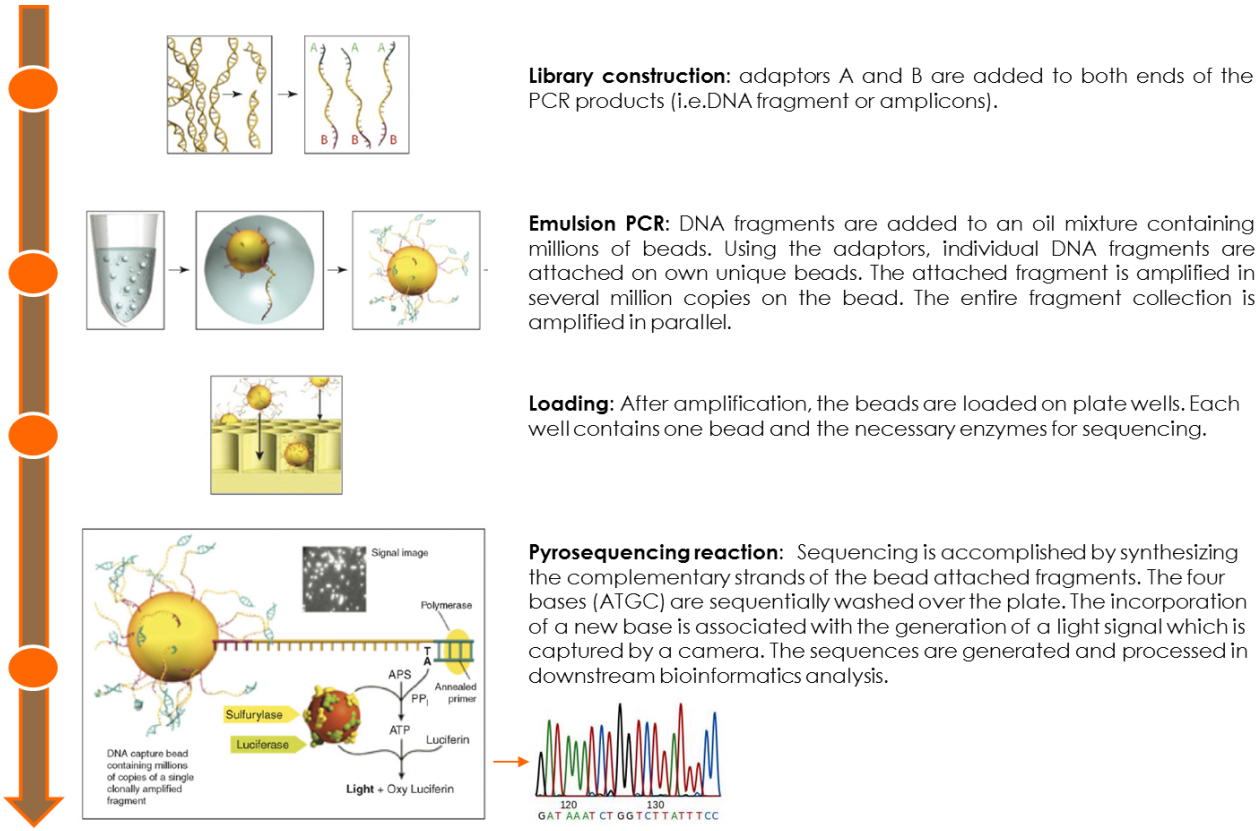
In our study, we used an amplicon-based sequencing approach to explore the taxonomic diversity of soil microbial communities. This molecular method is based on the analysis of DNA directly extracted from soil samples. In a nutshell, this method is based on the amplification of a genetic marker, which is a short gene fragment found in all organisms. In our study, we used the small subunit (SSU) 16S ribosomal gene (rDNA) for bacteria and the large subunit (LSU) 28S rDNA for fungi. The ribosomal gene features key characteristics that are required to be a good genetic marker: universally present in all organisms, featuring short variable regions of high information content (allow for species distinction), high conserved regions (to anchor primer), and very low rate of lateral gene transfer (stable in time).

DNA-amplicons, generated from the PCR amplification of the 16S bacterial and 28S fungal ribosomal gene, can be used in downstream analysis to characterize the taxonomic structure of microbial communities. In our study, we used the high-throughput 454-pyrosequencing technology, a next-generation sequencing technology that allow for the taxonomic identification of microorganisms at higher resolution, coverage and throughput that what was possible with older DNA sequencing technologies of first generation (e.g. Sanger) (Glenn, 2011). An overview of the different steps of the 454-pyrosequencing technology is presented in **Figure 14**, according to Mardis et al., (2008).

The raw *sequences* obtained are further processed in downstream bioinformatics analysis through three main steps that are: (1) curation of the sequences, (2) sequence clustering into operational taxonomic unit (OTU), and (3) taxonomic assignment (**Figure 13**).

Following the curation of the sequences, i.e. increasing the quality of data by removing notably the PCR- and sequencing-based errors, the sequences are clustered into OTUs, refers as individuals, based on their percentage of similarity. In our study, we used the standard 97% cutoff to cluster sequences into OTUs. This clustering approach allows one to deal with the “uncultured” and “unclassified” microbial sequences. In that way, these sequences that are usually highly abundant in soil ecosystems are considered in the total diversity. The assignment of each OTU to taxonomy, i.e. phylum, class, order, family, genus and “species” was performed by comparing sequences with sequences of reference from SILVA (Pruesse et al., 2007), one of available reference databases. The final table, containing information on the taxonomic assignment and the relative abundance of

each OTU in each sample, is used in downstream statistical analysis to explore the differences in microbial diversity among samples. In our study, we used multivariate statistical analysis to explore these differences including notably ordination methods and permutation tests (Anderson et al., 2006; Buttigieg and Ramette, 2014).



Library construction: adaptors A and B are added to both ends of the PCR products (i.e.DNA fragment or amplicons).

Emulsion PCR: DNA fragments are added to an oil mixture containing millions of beads. Using the adaptors, individual DNA fragments are attached on own unique beads. The attached fragment is amplified in several million copies on the bead. The entire fragment collection is amplified in parallel.

Loading: After amplification, the beads are loaded on plate wells. Each well contains one bead and the necessary enzymes for sequencing.

Pyrosequencing reaction: Sequencing is accomplished by synthesizing the complementary strands of the bead attached fragments. The four bases (ATGC) are sequentially washed over the plate. The incorporation of a new base is associated with the generation of a light signal which is captured by a camera. The sequences are generated and processed in downstream bioinformatics analysis.

Figure 14 454-pyrosequencing workflow (adapted from Mardis et al., (2008)). The library construction ligates adaptors to DNA fragments that are further attached on beads and amplified millions of times in emulsion PCR. The beads are loaded into plate wells with enzymes for sequencing. The pyrosequencing reaction is based on the emission of a light signal when a new base (ACGT) is incorporated. The output sequences are further processed in downstream bioinformatics analysis.

6. References

Aislabie, J., Deslippe, J.R., Dymond, J., others, 2013. Soil microbes and their contribution to soil services. Ecosyst. Serv. N. Z. Cond. Trends 143161.

Allison, S.D., Hanson, C.A., Treseder, K.K., 2007. Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. Soil Biol. Biochem. 39, 1878–1887. doi:10.1016/j.soilbio.2007.02.001

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. Agric. Ecosyst. Environ. 74, 19–31.

Anderson, M.J., Ellingsen, K.E., McArdle, B.H., 2006. Multivariate dispersion as a measure of beta diversity. Ecol. Lett. 9, 683–693.

Assessment, M.E., 2005. Millennium ecosystem assessment. Ecosyst. Hum. Well- Biodivers. Synth. Publ. World Resour. Inst. Wash. DC.

Barrios, E., 2007. Soil biota, ecosystem services and land productivity. Ecol. Econ. 64, 269–285.

Basch, G., Friedrich, T., Kassam, A., Gonzalez-Sanchez, E., 2015. Conservation Agriculture in Europe, in: Farooq, M., Siddique, K.H.M. (Eds.), Conservation Agriculture. Springer International Publishing, Cham, pp. 357–389.

Bommarco, R., Kleijn, D., Potts, S.G., 2013. Ecological intensification: harnessing ecosystem services for food security. Trends Ecol. Evol. 28, 230–238. doi:10.1016/j.tree.2012.10.012

Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27, 325–349.

Buttigieg, P.L., Ramette, A., 2014. A guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses. FEMS Microbiol. Ecol. 90, 543–550. doi:10.1111/1574-6941.12437

Carbonetto, B., Rascovan, N., Álvarez, R., Mentaberry, A., Vázquez, M.P., 2014. Structure, Composition and Metagenomic Profile of Soil Microbiomes Associated to Agricultural Land Use and Tillage Systems in Argentine Pampas. PLoS ONE 9, e99949. doi:10.1371/journal.pone.0099949

Cardenas, E., Tiedje, J.M., 2008. New tools for discovering and characterizing microbial diversity. Curr. Opin. Biotechnol. 19, 544–9. doi:10.1016/j.copbio.2008.10.010

Cleveland, C.C., Townsend, A.R., Schimel, D.S., Fisher, H., Howarth, R.W., Hedin, L.O., Perakis, S.S., Latty, E.F., Von Fischer, J.C., Elseroad, A., others, 1999. Global patterns of terrestrial biological nitrogen (N2) fixation in natural ecosystems. Glob. Biogeochem. Cycles 13, 623–645.

Colinet, G., Bergen, J.-C., Lemtiri, A., 2013. Projet SOLRESIDUS: caractérisation pédologique, variabilité spatiale et dynamique temporelle de la disponibilité en éléments nutritifs. (Rapport interne), ULg, Gembloux Agro Biotech.

Degrune, F., Dufrêne, M., Colinet, G., Massart, S., Taminiau, B., Bodson, B., Hiel, M.-P., Daube, G., Nezer, C., Vandenbol, M., 2015. A novel sub-phylum method discriminates better the impact of crop management on soil microbial community. Agron. Sustain. Dev. 1–10.

Doré, T., Makowski, D., Malézieux, E., Munier-Jolain, N., Tchamitchian, M., Tittonell, P., 2011. Facing up to the paradigm of ecological intensification in agronomy: revisiting methods, concepts and knowledge. Eur. J. Agron. 34, 197–210.

Dorr de Quadros, P., Zhalnina, K., Davis-Richardson, A., Fagen, J.R., Drew, J., Bayer, C., Camargo, F.A., Triplett, E.W., 2012. The effect of tillage system and crop rotation on soil microbial diversity and composition in a subtropical Acrisol. Diversity 4, 375–395. doi:10.3390/d4040375

East, R., 2013. Microbiome: Soil science comes to life. Nature 501, S18–S19.

Fierer, N., Breitbart, M., Nulton, J., Salamon, P., Lozupone, C., Jones, R., Robeson, M., Edwards, R.A., Felts, B., Rayhawk, S., Knight, R., Rohwer, F., Jackson, R.B., 2007. Metagenomic and Small-Subunit rRNA Analyses Reveal the Genetic Diversity of Bacteria, Archaea, Fungi, and Viruses in Soil. Appl. Environ. Microbiol. 73, 7059–7066. doi:10.1128/AEM.00358-07

Foley, J.A., 2005. Global Consequences of Land Use. Science 309, 570–574. doi:10.1126/science.1111772

Glenn, T.C., 2011. Field guide to next-generation DNA sequencers. Mol. Ecol. Resour. 11, 759–769. doi:10.1111/j.1755-0998.2011.03024.x

Hatt, S., Artru, S., Brédart, D., Lassois, L., Francis, F., Haubruge, É., Garré, S., M. Stassart, P., Dufrêne, M., Monty, A., Boeraeve, F., 2016. Towards sustainable food systems: the concept of agroecology and how it questions current research practices. A review. BASE.

Hobbs, P.R., Sayre, K., Gupta, R., 2008. The role of conservation agriculture in sustainable agriculture. Philos. Trans. R. Soc. B Biol. Sci. 363, 543–555. doi:10.1098/rstb.2007.2169

Jiménez-Bueno, N.G., Valenzuela-Encinas, C., Marsch, R., Ortiz-Gutiérrez, D., Verhulst, N., Govaerts, B., Dendooven, L., Navarro-Noya, Y.E., 2016. Bacterial indicator taxa in soils under different long-term agricultural management. J. Appl. Microbiol. n/a-n/a. doi:10.1111/jam.13072

Jung, J., Philippot, L., Park, W., 2016. Metagenomic and functional analyses of the consequences of reduction of bacterial diversity on soil functions and bioremediation in diesel-contaminated microcosms. Sci. Rep. 6. doi:10.1038/srep23012

Kibblewhite, M.G., Ritz, K., Swift, M.J., 2008. Soil health in agricultural systems. Philos. Trans. R. Soc. Lond. B Biol. Sci. 363, 685–701. doi:10.1098/rstb.2007.2178

Lemanceau, P., Maron, P.-A., Mazurier, S., Mougel, C., Pivato, B., Plassart, P., Ranjard, L., Revellin, C., Tardy, V., Wipf, D., 2014. Understanding and managing soil biodiversity: a major challenge in agroecology. Agron. Sustain. Dev. 35, 67–81. doi:10.1007/s13593-014-0247-0

Mardis, E.R., 2008. The impact of next-generation sequencing technology on genetics. Trends Genet. TIG 24, 133–41. doi:10.1016/j.tig.2007.12.007

Montgomery, D.R., 2007. Soil erosion and agricultural sustainability. Proc. Natl. Acad. Sci. 104, 13268–13272.

Monty, A., Garré, S., Bindelle, J., Mahy, G., Deleu, M., Bodson, B., Haubruge, E., 2016. AgricultureIsLife or how to facilitate innovation in agriculture through multi-disciplinary research? Biotechnol. Agron. Société Environ. 20, 211–214.

Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670.

Navarro-Noya, Y.E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M.C., Valenzuela-Encinas, C., Jiménez-Bueno, N., Verhulst, N., Govaerts, B., Dendooven, L., 2013. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biol. Biochem. 65, 86–95. doi:10.1016/j.soilbio.2013.05.009

Philippot, L., Spor, A., Hénault, C., Bru, D., Bizouard, F., Jones, C.M., Sarr, A., Maron, P.-A., 2013. Loss in microbial diversity affects nitrogen cycling in soil. ISME J. 7, 1609–1619. doi:10.1038/ismej.2013.34

Pittelkow, C.M., Liang, X., Linquist, B.A., van Groenigen, K.J., Lee, J., Lundy, M.E., van Gestel, N., Six, J., Venterea, R.T., van Kessel, C., 2014. Productivity limits and potentials of the principles of conservation agriculture. Nature 517, 365–368. doi:10.1038/nature13809

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35, 7188–7196. doi:10.1093/nar/gkm864

Reicosky, D.C., 2015. Conservation tillage is not conservation agriculture. J. Soil Water Conserv. 70, 103A–108A. doi:10.2489/jswc.70.5.103A

Rodríguez, H., Fraga, R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17, 319–339.

Roesch, L.F.W., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., Daroub, S.H., Camargo, F.A.O., Farmerie, W.G., Triplett, E.W., 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J. 1, 283–290. doi:10.1038/ismej.2007.53

Schloter, M., Dilly, O., Munch, J.., 2003. Indicators for evaluating soil quality. Agric. Ecosyst. Environ. 98, 255–262. doi:10.1016/S0167-8809(03)00085-9

Sengupta, A., Dick, W.A., 2015. Bacterial Community Diversity in Soil Under two Tillage Practices as Determined by Pyrosequencing. Microb. Ecol. 70, 853–859. doi:10.1007/s00248-015-0609-4

Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Sci. Soc. Am. J. 63, 1350–1358.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. Soil Sci. Soc. Am. J. 70, 555. doi:10.2136/sssaj2004.0347

Smith, B., Wilson, J.B., 1996. A consumer's guide to evenness indices. Oikos 70–82.

Smith, K.A., McTaggart, I.P., Tsuruta, H., 1997. Emissions of N2O and NO associated with nitrogen fertilization in intensive agriculture, and the potential for mitigation. Soil Use Manag. 13, 296–304.

Smith, V.H., Tilman, G.D., Nekola, J.C., 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut. 100, 179–196.

Soliveres, S., van der Plas, F., Manning, P., Prati, D., Gossner, M.M., Renner, S.C., Alt, F., Arndt, H., Baumgartner, V., Binkenstein, J., Birkhofer, K., Blaser, S., Blüthgen, N., Boch, S., Böhm, S., Börschig, C., Buscot, F., Diekötter, T., Heinze, J., Hölzel, N., Jung, K., Klaus, V.H., Kleinebecker, T., Klemmer, S., Krauss, J., Lange, M., Morris, E.K., Müller, J., Oelmann, Y., Overmann, J., Pašalić, E., Rillig, M.C., Schaefer, H.M., Schloter, M., Schmitt, B., Schöning, I., Schrumpf, M., Sikorski, J., Socher, S.A., Solly, E.F., Sonnemann, I., Sorkau, E., Steckel, J., Steffan-Dewenter, I., Stempfhuber, B., Tschapka, M., Türke, M., Venter, P.C., Weiner, C.N., Weisser, W.W., Werner, M., Westphal, C., Wilcke, W., Wolters, V., Wubet, T., Wurst, S., Fischer, M., Allan, E., 2016. Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. Nature 536, 456–459. doi:10.1038/nature19092

Tilman, D., 1999. Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proc. Natl. Acad. Sci. 96, 5995–6000.

Tilman, D., 1998. The greening of the green revolution. Nature 396, 211–212.

Titi, A.E., 2002. Soil Tillage in Agroecosystems. CRC Press.

Urich, T., Lanzén, A., Qi, J., Huson, D.H., Schleper, C., Schuster, S.C., 2008. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PloS One 3, e2527.

van der Heijden, M.G. a, Bardgett, R.D., van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11, 296–310. doi:10.1111/j.1461-0248.2007.01139.x

Van Der Heijden, M.G., Bakker, R., Verwaal, J., Scheublin, T.R., Rutten, M., Van Logtestijn, R., Staehelin, C., 2006. Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. FEMS Microbiol. Ecol. 56, 178–187.

Van der Heijden, M.G., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 69–72.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7, 737–750.

Whittaker, R.H., 1972. Evolution and measurement of species diversity. Taxon 213–251.

Zhang, W., Ricketts, T.H., Kremen, C., Carney, K., Swinton, S.M., 2007. Ecosystem services and dis-services to agriculture. Ecol. Econ. 64, 253–260.

7. Supplementary material

Date	Operation field	Plot
2012		
29/08	Shallow tillage	All
06/09	Cover crop sowing (mustard)	All
13/12	plowing	CT

Date	Operation field	Plot
2013		
18/03	Weeding	All
05/04	Sowing faba bean	All
08/04	Meadow-emergence weeding	All
15/04	Soil sampling	All
24/05	Soil sampling	All
27/06	Soil sampling	All
08/07	Chemical pest control	All
28/08	Weeding	All
04/09	Faba bean harvest	All
25/11	Plowing	CT
25/11	Shallow tillage	All
25/11	Sowing winter wheat	All

Date	Operation field	Plot
2014		
11/03	Nitrogen fertilization	All
26/03	Soil sampling	All
01/04	Weeding	All
15/04	Nitrogen fertilization	All
15/04	Growth regulator	All
25/04	Weeding	All
27/04	Fungicide	All
12/05	Nitrogen fertilization	All
16/05	Weeding	All
06/06	Fungicide	All
14/07	Soil sampling	All
04/09	Winter wheat harvest	All

S1 Dates of field operations on SOLRESIDUS in 2012, 2013 and 2014. In brown text; the different phytosanitary treatments, in blue; the nitrogen fertilization, and in green; the soil sampling.

Chapter II. Crop residue management in temperate climate: A review

Crop residue management in arable cropping systems under temperate climate. Part 1: Soil biological and chemical (phosphorus and nitrogen) properties. A review

Published in the special issue *AgricultureIsLife or how to facilitate innovation in agriculture through multi-disciplinary research?* in *Biotechnologie, Agronomie, Société et Environnement*, 2016, 20 (s1).

Aboulkacem Lemtiri*, **Florine Degrune***, Sophie Barbieux, Marie-Pierre Hiel, Marie Chélin, Nargish Parvin, Micheline Vandenbol, Frédéric Francis, Gilles Colinet

** These authors have contributed equally to this work*

Outline

The full publication aimed to review the state of art regarding effects of crop residue management under different tillage regimes in a temperate climate. In this first part, we focus on soil biological and chemical properties. This review was a collaborative work carried out in the context of the AgricultureIsLife research platform.

1. Abstract

Interacting soil organisms support biological processes that participate in soil functions, organic matter decomposition, and nutrient cycling. Earthworms and microorganisms provide a range of beneficial roles in agricultural systems, including increased organic matter mineralization, nutrient cycling, and soil structure stabilization.

The following aspects of crop residue management effects were examined in this paper: (i) earthworm community composition and structure; (ii) soil microbial communities; and (iii) phosphorus and nitrogen element availability and distribution in the soil profile. Conventional tillage (ploughing) is often reported to generate decreased soil organism abundance and diversity, primarily earthworms and microorganisms, as well as a uniform distribution of nutrients (P and N) within the ploughed soil horizon. Soil residue incorporation of mineral particles can maintain P and N levels, however returning soil also increases aeration and activation of microbial activity. Hence, comparisons of tillage effects on soil biological functioning and nutrient cycling remain unclear.

This review highlights the challenges in establishing definitive evidence regarding the effects of crop residue management on soil organisms and nutrient dynamics. The studies examined reported variability in soil and climate, and the complexity of soil processes contributed to the absence of clear findings. Further research is required under temperate climate conditions.

Keywords

Crop residue management, tillage, earthworms, microorganisms, phosphorus, nitrogen; temperate climate

2. Introduction

Soil organic matter (OM) serves a key role in soil fertility under agricultural practices. It is an important source of crop nutrients, as well as a nutrient source for the high soil biodiversity levels. Soil macro- and microorganisms are involved in key biological processes, such as carbon dynamics and nutrient cycling (Wardle, 1995). More specifically, earthworms and microorganisms provide a range of beneficial roles, including SOM mineralization, soil aggregate generation and stabilization, and nutrient stimulation (Lemtiri et al., 2014). However, environmental factors, such as climate, as well as anthropogenic activities, particularly agricultural management practices influence soil biological communities and their functions at different levels. In most soils, >90% of the total nitrogen (N) and sulphur (S), together with >50% of the total phosphorus (P) are associated with microbial biomass and OM, therefore cycling and bioavailability of these key soil nutrients are primarily controlled by OM transformation associated with microbial and faunal activity (Bünemann and Condron, 2007; McNeill and Unkovich, 2007). Anthropogenic impacts have dramatically altered global nutrient cycles; in some regions, ecosystems suffer nutrient excesses due to uncontrolled run-off of anthropogenically derived N and P (Vitousek, 2004), whilst in other areas, crop production is limited by lack of these soil elements.

Agriculture also faces the growing demand for bio-products production on cultivated lands. Questions addressing best crop residue use, i.e. off-site valorisation, simply left at the topsoil (no-till and reduced tillage systems) or mixed within the soil profile using ploughing (conventional tillage), and soil resilience to changes in input/output balance are continually addressed and answers should integrate entire soil functioning components. Therefore, we focused this review on the impacts of tillage practices, including conventional and minimum or zero tillage, crop residue management on biological functioning, and P and N cycling. An additional paper (Hiel et al., 2016) examines the hydrological cycle and crop performance.

3. Literature

Effects of tillage systems and crop residue incorporation on microorganisms and earthworms

In the following section, we focus on the effects of tillage and crop residue incorporation on soil microorganisms, the most abundant and diverse group of soil organisms (Roesch et al., 2007); and earthworms, the soil macrofauna integrally involved in soil aggregation processes. Both organisms are well studied and support essential soil ecosystem functions, such as nutrient and soil carbon cycling, disease suppression, organic matter degradation, and soil structure (Wardle, 1995). Holland (2004) reviewed the consequences of conservation agriculture, including crop residue incorporation and application of minimum or zero tillage on global soil biology. Earthworms are known as ecosystem engineers due to the species profound impacts on soil habitat; earthworms change soil chemical, physical, and structural properties, which subsequently impact soil biota and ecosystem functioning. Earthworms and microorganisms are both functional groups, which interact. Earthworms are highly involved in the biochemical decomposition of OM, where they indirectly stimulate microbial activity through fragmentation and ingestion of fresh OM, resulting in increased surface area available for microbial colonisation, which in turn produce enzymes causing OM decomposition (Curry and Schmidt, 2007). Therefore, the notable relationship between microorganisms and earthworms is of agronomical interest and is essential to assess the impacts of tillage and crop residue incorporation on earthworm biomass, activity, and community composition.

Earthworms

Land management practices have considerable impact on earthworm community size and dynamics. Agriculture intensification has included various chemical and mechanical applications, often with little consideration of the effects on biologically mediated processes. Organic and inorganic fertiliser sources influence soil fertility, but OM is particularly important as it provides the soil more resilience by smoothing inter-annual variation in nutrient availability for soil biota and crop productivity (Palm et al., 1997).

Agricultural practices, such as tillage alter soil microhabitats and interrupt life cycles. Therefore, it is expected that soil organisms with long life spans (i.e. up to six years under optimum conditions) are

particularly sensitive, such as earthworms (Eriksen-Hamel et al., 2009). Van Capelle et al. (2012) reported a decline in earthworm species diversity due to frequent tillage, which affected soil physical properties with detrimental effects to many soil organisms. For example, tillage affects earthworm populations, which build their galleries and burrows in deeper soil layers. Johnson-Maynard et al. (2007) found intensive and frequent tillage markedly reduced earthworm populations, whilst conservation tillage systems (no-till) promoted an increase in populations. Tillage not only affected earthworm abundance and biomass, but also induced changes in ecological groups and species diversity. Simonsen et al. (2010) showed reduced tillage and manure use positively affected anecic earthworms, whereas conventional tillage practices appeared to benefit endogeic species. Higher earthworm number and biomass in conservation tillage agrosystems were attributed to surface litter, SOM accumulation, favourable pedoclimatic conditions, and reduction in disturbance regimes (Nuutinen, 1992; Wyss and Glasstetter, 1992). However, in some cases, Kladivko et al. (2001) indicated earthworm abundance and biomass might be equal or slightly lower in no-till compared with conventional tillage agrosystems. One factor responsible for this inconsistency is tillage often occurs in conjunction with the incorporation of crop residues, which are food sources for earthworms.

Tillage systems more rapidly affect some earthworm ecological groups relative to others. Clear ploughing effects were demonstrated for anecic and epigeic species, as these two groups require soil surface litter and cannot tolerate regular habitat disturbance. Studies showed endogeic earthworms exhibited reduced sensitivity to soil inversion and were less impacted by tillage systems; endogeic earthworms were even sometimes favoured by ploughing, as access to OM was facilitated when crop residues were buried and partially decomposed by soil microorganisms (Nuutinen, 1992; Wyss and Glasstetter, 1992). The endogeic earthworm A. caliginosa was considered to be tolerant of soil tillage (Peigné et al., 2009; Rosas-Medina et al., 2010), although de Oliveira et al. (2012) found it to be more sensitive to tillage than A. rosea. Berner et al. (2008) reported ploughing decreased endogeic species abundance. Ploughing with various conservation tillage systems was compared and results showed the number of adult endogeic earthworms was 70% higher for conservation tillage, whilst total biomass was 50% lower and individual biomass under conservation tillage was only one-third that under ploughing (Berner et al., 2008). The authors argued food resources were more favourable for endogeic worms in ploughed plots than in plots subjected to conservation tillage. However, Chan (2001) examined the impacts of

conventional tillage practices on earthworm population density and reported conflicting results. Therefore, depending on quality and quantity of residues incorporated in the soil versus that left on the surface, tillage might inhibit or enhance earthworm populations (Chan, 2001; Zaller and Köpke, 2004). Cropping systems where cereals are under-sown with legumes support higher earthworm number and biomass than those with monoculture cereals (Schmidt et al., 2003). This could be due to tillage reduction, higher organic matter input, or higher quality residue composition following a cereal-legume intercrop, which might be more favourable to earthworms. The importance of the factors was investigated in a field experiment at Long Ashton Research Station, UK, where earthworm populations under conventional wheat, direct-drilled wheat, and direct-drilled wheat-clover intercrops were compared (Schmidt et al., 2003). The results indicated the following: (i) the absence of ploughing alone had only a modest effect on earthworm populations; and (ii) the combination of ploughing and presence of a clover understory substantially increased earthworm populations. Schmidt et al. (2003) concluded earthworm populations exhibited decreased benefit from reduced soil disturbance relative to the enhanced quantity, nutritional quality, and continuity of food supply in wheat-clover intercrops.

Earthworm growth and reproduction are often limited by food availability in agricultural soils. Therefore, cropping regimes influence earthworm community structure and population density (Shuster et al., 2003). In conventional tillage systems, crop residues are incorporated into the soil, which might increase residue availability for earthworms feeding at the soil surface. Fortune et al. (2005) established a field experiment to investigate the effects non-inversion tillage vs. conventional tillage and straw chopping vs. baling and removing on earthworms. After four years, earthworm abundance and biomass increased in conservation tillage, where straw was incorporated. However, earthworm population response to crop residues depended primarily on the residue biochemical characteristics. Earthworm populations were larger in long term conservation compared with conventional tillage plots, however crop residue management did not affect earthworm populations, possibly due to the high C: N ratio of straw.

Microorganisms

Soil microbial communities, including bacteria and fungi, are other important actors in maintaining soil ecosystem functioning and sustainability (Paul, 2014; Singh et al., 2011). Organic matter is the main nutrient source for soil organisms, thus adding or not adding organic products (animal- or plant-based) to soil is expected to have an effect on the microbial properties.

Kallenbach and Grandy (2011) conducted a meta-analysis of 41 studies to assess microbial biomass responses to diverse types of organic amendments (solid, raw, or composted animal derived materials, and plant derived residues) relative to systems that received only inorganic fertiliser applications. The meta-analysis included a broad range of soil types, cropping systems, and geographic locations. Results showed climate and edaphic soil properties, as well as crop and soil management practices imposed a diverse range of constraints on soil microbial biomass responses to organic amendments, but in all studies, adding organic amendments led to significant increases in microbial biomass.

In addition to global positive effects of organic amendments on microbial biomass, interest in how crop residue location in the soil profile impacts microbial properties is represented in the literature. Franzluebbers (2002) indicated conventional tillage restricted the organic amendment to the plough layer, whilst conservation tillage systems, such as zero- or reduced-tillage applied the crop residue at or close to the soil surface generated a decreasing gradient of residue content from surface to depth.

Given the vertical gradient observed in conservation tillage and the close dependence of microbial communities on resource quality and availability, differences in microbial properties between conservation and conventional tillage systems can be expected. For example, Fierer (2003) reported soil microbial biomass decreased with depth and tillage practice effects on microbial biomass were more pronounced at the soil surface compared with deeper horizons. Globally, conservation tillage systems, such as zero-tillage increased microbial biomass relative to conventional tillage practices in long-term field experiments (Helgason et al., 2009, 2010; Shi et al., 2013; van Groenigen et al., 2010; Wang et al., 2012).

Furthermore, in addition to biomass, the relationship between functional microbial group structures, including bacteria, fungi and arbuscular mycorrhizal fungi (AMF) and crop residue localisation have been investigated by several methods, such as phospholipid fatty acid profiles (PLFA). For example, Wang et al. (2012) showed tillage system impacts on microbial structure; conservation tillage increased AMF and conventional tillage increased bacteria. Results indicted conventional tillage significantly decreased soil fungi by physically disrupting fungal hyphal networks. However, Helgason et al. (2010) reported crop residue placement did not influence microbial community structure.

Höflich et al. (1999) conducted a survey to investigate conservation vs. conventional tillage impacts in two soil types (sandy loam and loamy sand). The study examined the effects on specific microbial group distribution and activity in the rhizosphere (*Rhizobium spp*., mycorrhizal species, *Pseudomonas spp*.) serving integral roles in agriculture. Results suggested conservation tillage stimulated rhizosphere bacteria, particularly *Agrobacterium spp*. and *Pseudomonas spp*. Nodulation and N2 fixation from *Rhizobium spp*. also increased, but only in sandy loam. Finally, rhizosphere colonisation differences by mycorrhizal and saprophytic fungi between both tillage practices were not observed.

More recently, the discipline has benefited from rapid developments in massive parallel DNA sequencing technologies (New Generation Sequencing-NGS), which provide quick and deep sequencing of metagenomic DNA at moderate costs, allowing detailed assessments of soil microbial communities with higher phylogenetic resolution, which provide more taxonomic information than previous classical sequencing techniques (e.g., Sanger) requiring notably more time and money (Cardenas and Tiedje, 2008). A few surveys to date investigated the influence of crop residue utilisation combined with different tillage systems using massive parallel DNA sequencing (Ceja-Navarro et al., 2010; Degrune et al., 2015; Navarro-Noya et al., 2013a). Degrune et al. (2015) reported significant effects of tillage practices on bacterial and fungal community composition, while influences of crop residue utilisation were not observed. Navarro-Noya et al. (2013), however, found crop residue utilisation and tillage practice impacts on different bacterial groups. Ceja-Navarro et al. (2010) showed some functional bacterial groups, such as fluorescent *Pseudomonas spp.* and Burkholderiales were favoured by residue incorporation and negatively affected by residue removal. As compared to the experiment of Degrune et al. (2015), located in a temperate climate region, others have been conducted under semi-arid condition. This could explain the differences observed about the influence of crop residue management and tillage.

Nutrient availability: Phosphorus and Nitrogen

Phosphorus (P) and nitrogen (N) are major crop nutrients, which must be added to soils when levels are insufficient to ensure acceptable crop production (i.e., yield). Indeed, if OM mineralisation by microorganisms is a key process in making P and N available for plant nutrition, mineral fertilisers can be applied as directly available nutrient sources. However, unmanaged chemical fertiliser use often generates environmental and economic concerns. For example,

chemical fertiliser production is derived from non-renewable resources, including fossil fuels (Cordell et al., 2009). In addition, P and N application can result in eutrophication of surface water, including but not limited to lakes, rivers, and estuaries or leaching towards groundwater and subsequent contamination (Carpenter et al., 1998). Therefore, appropriate N and P management is critical to ensure agricultural sustainability with minimal negative environmental impacts.

Clearly, benefits and concerns surround the potential to apply P and N as a nutrient source in plants, resulting in interest to optimise application management. Crop residues show potential for the following valorisation schemes: animal feeding, energy production, construction materials, biosourced molecules, and of course soil fertility conservation. Presently, studies have not shown the compatibility level between crop residue exportation and sustainable soil health.

Phosphorus

P is a limiting nutrient for biological productivity in numerous ecosystems. Most agrosystems rely on mineral P-fertilisers and organic manure applications, even under widely variable conditions worldwide, but including temperate regions, where we focus our review. Organic (P_{orga}) and inorganic (P_{inorga}) phosphorus compose a respective 30 to 65% and 35 to 70% total phosphorus (P_{tot}) in soils (Harrison, 1987). This chapter specifically examines the question of soil crop residue management impacts through restitution *vs.* exportation and soil tillage.

The following points should be emphasised regarding P: (i) 96% of the P taken up by plants is derived from dissolved forms ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) (Beck and Sanchez, 1994); (ii) inorganic phosphorus is weakly available to plants due to low mineral solubility and strong interactions (sorption, precipitation) with soil constituents (e.g., 9% of P_{tot} in Belgium soils; Renneson et al. (2013)); (iii) phosphate rocks of high quality are expected to disappear in the upcoming decades (Cordell et al., 2009); and (iv) mobilisation of organic P sources in soils strongly relies on factors governing OM mineralisation.

Crop residues contain inorganic and organic P forms, easily available for plants and microorganisms (Noack et al., 2012). Orthophosphates (PO_4^{3-}) are dominant in crop residues, which can be directly taken up by plants, immobilised by microorganisms, or sorbed to soil minerals. Noack et al. (2012) reported P_{orga} from residues can be mineralised to P_{inorga} and subsequently stabilised, reducing P_{orga} availability. Nevertheless, crop residue incorporation effects on soil P mobility remain unclear and results of studies show a lack of congruency. Ohno and Erich (1997) observed the release of

dissolved organic carbon from crop residues in acidic soils, inhibiting P adsorption rates onto soils. This was due to Aluminium (Al) surface complexation by dissolved OM ligands and desorption of the complex into soil solution, resulting in increased soil P availability. However, Varinderpal-Singh et al. (2006) reported crop residue incorporation in neutral soils increased maximum P adsorption, as well as resistance to P release in soil solution, resulting in decreased P desorption. Wang et al. (2011) found increased P_{tot} content with residue incorporation; decreased P_{orga}, and no change in available P (P_{av}). The study concluded residue had no effects on P content under conventional tillage. Soil reaction constituted an important factor, as different P immobilisation effects occurred based on soil pH. However, processes were also dependent on residue characteristics (type, quantity, and C: P ratio, among others), soil P richness, and environmental factors (e.g., climatic conditions, soil properties).

Tillage practices can also influence P release from crop residues. Coppens et al. (2006) showed mixing crop residues with soil particles by mouldboard plough practices resulted in accelerated residue decomposition and subsequently increased nutrient release. Wang et al. (2011) showed P_{tot} and P_{orga} contents were higher under conservation compared with conventional tillage treatments, whereas significant differences in P_{av} were not observed.

Deubel et al. (2011) showed the influence of tillage treatments on element spatial distribution, which included P within the soil profile. Evidence indicated crop residues under conventional tillage were distributed uniformly throughout the plough layer. However, under a conservation tillage system, residues were not incorporated in the plough layer and decomposed at the soil surface resulting in OM accumulation, which released P in the soil surface layer. This accumulation was heightened by P amendments via inorganic fertiliser or animal manure applications (Sharpley, 2003).

Nevertheless, tillage treatments as such exhibited minimal effects on P_{tot} content. Piegholdt et al. (2013) reported a slight increase in soil P content under a conservation tillage system compared with conventional tillage in German Luvisols. However, Sharpley (2003) indicated ploughing increased soil P retention under conservation tillage practices. In addition, maximum P sorption and minimum P sorption saturation was observed by mixing topsoil (0-5 cm) and subsoil (5-20 cm) (Sharpley, 2003).

Nitrogen

Nitrogen is one of the most deficient nutrients in agricultural soils and hence the major fertiliser used in agriculture. Nitrogen can be found in organic and inorganic forms. Organic N availability is slow compared to inorganic, but the risks of N escape from soils to other environmental components are also reduced. The primary N forms available for plant nutrition are nitric (NO3-N) and ammonium (NH4-N). Crop residues are the main organic source of soil nutrients, and residue return was shown to enhance soil nitrogen stocks (Dolan et al., 2006; Malhi et al., 2006, 2010).

Chen et al. (2014) found the following four pathways were responsible for soil inorganic nitrogen conditioning: (i) biotic immobilisation/remineralisation by microbes; (ii) abiotic immobilisation into dissolved organic or recalcitrant N; (iii) soil organic matter mineralisation; and (iv) organic plant residue mineralisation. Returning crop residues to soil should have direct effects on the fluxes from pathway (iv), but should also improve the immobilisation rates into microbial biomass, remineralisation rate from microbes, and microbe mortality.

Chen et al. (2014) found residue decay rates were generally slower at the surface under conservation practices than when incorporated in the soil under conventional tillage, where N release was delayed from residues under conservation tillage systems. However, crop residue placement effects within the soil profile using tillage practice on N content remain unclear. Dolan et al. (2006) conducted a survey under continental climate conditions (Minnesota, USA) and found the absence of tillage effects for the entire soil profile (0-45 cm), however effects were observed for specific soil profile layers. At the soil surface (0-15 cm), N content was higher under conservation tillage; the 15-20 cm depth was considered a transition zone; and at 20 cm and below, N content was higher under ploughed soil. Angers et al. (1997) in eastern Canada reached the same conclusions. However, Brennan et al. (2014) conducted a survey under a cool Atlantic climate in Ireland between 2009 and 2011 and concluded N response was similar for conventional and conservation tillage, and crop residue utilisation (returned to soil or removed) had very little effect on plant N uptake.

Malhi et al. (2010) conducted a field experiment in Alberta, Canada and demonstrated a combination of returning crop residues and conservation tillage increased the total and light fraction N content in the 0-15 cm soil layer compared with conventional tillage. However, differences were not observed for NO_3-N content. Thus, N response to crop residue management

might change with the N form under consideration. Chen et al. (2014) suggested ploughing time is also a factor to include.

Furthermore, in addition to crop residue utilisation, it is important to examine crop residue composition. A low C: N ratio (e.g., residues from legume crops) will generally induce residue mineralisation by microbes, whereas a high C: N ratio will cause immobilisation by microbes; consequently N is no longer available for plant nutrition (Christopher and Lal, 2007). Morris et al. (2010) and Chen et al. (2014) report returning cereal straws to soils without tillage can promote a negative effect on the next generation of crops due to immobilisation of mineral N by microbes. However, Crops that are able to fix nitrogen from atmosphere due to the presence of specific group of microorganisms (*rhizobium* sp.) or crops with long growth period are less sensitive to this phenomenon (Chen et al., 2014).

4. Conclusion

The fate of crop residues (exportation, incorporation, one or the other system) is crucial in earthworm and microbial community viability, and P and N availability are essential soil fertility elements. Soil macro- and microorganisms, including earthworms and microbial communities are potentially key indicators of soil health. These organisms respond rapidly to changes in soil conditions, such as crop residue management and tillage systems. Overall, under most conditions, incorporating crop residues into the soil, which is the main nutrient source for soil biota had favourable effects on earthworms and microbial communities. However, burying crop residues using ploughing systems exhibited harmful effects on soil earthworms and microorganisms, such as fungi communities. Ploughing induced increased disturbance to larger organisms, such as earthworms and fungi, while bacteria located in soil micropores were less affected by ploughing. Field experiments also showed divergent effects of crop residue incorporation and tillage systems on soil P levels and forms. However, the evolution of soil nutrient content was clearly dependent on a balance of inputs *vs.* outputs. Tillage influenced P content and spatial distribution within the soil profile. Ploughing generated P accumulation in the soil surface layer. Crop residues that remained on fields and decomposed at the soil surface heightened P accumulation. Therefore, reduced tillage and residue conservation might improve P availability. However, based on the lack of congruence from various studies regarding crop residue and tillage treatment effects on soil P dynamics, further studies are required.

A clear positive effect of leaving residues, when combined with reduced tillage, increased soil N stock. In all cases, leaving crop residues on the field increased soil N content, while the effects of tillage changed according to residue localisation depth. It is vital to consider residue composition, as high C: N ratio generally causes microbial immobilisation and low C: N ratio favours mineralisation and N availability for crops. Research provides evidence that crop residue management and tillage system influences soil biological activity and nutrient cycling, which differ according to experiment location and duration. In this review, we examined experiments conducted under temperate climate; however we did not consider factors such as soil type, which can influence biotic activity and therefore the processes that change nutrient availability.

5. References

Angers, D.A., Bolinder, M.A., Carter, M.R., Gregorich, E.G., Drury, C.F., Liang, B.C., Voroney, R.P., Simard, R.R., Donald, R.G., Beyaert, R.P., Martel, J., 1997. Impact of tillage practices on organic carbon and nitrogen storage in cool, humid soils of eastern Canada. Soil Tillage Res., Soil Biology and Tillage 41, 191–201. doi:10.1016/S0167-1987(96)01100-2

Beck, M.A., Sanchez, P.A., 1994. Soil phosphorus fraction dynamics during 18 years of cultivation on a typic paleudult. Soil Sci. Soc. Am. J. 58, 1424–1431.

Berner, A., Hildermann, I., Fließbach, A., Pfiffner, L., Niggli, U., Mäder, P., 2008. Crop yield and soil fertility response to reduced tillage under organic management. Soil Tillage Res. 101, 89–96. doi:10.1016/j.still.2008.07.012

Brennan, J., Hackett, R., McCabe, T., Grant, J., Fortune, R.A., Forristal, P.D., 2014. The effect of tillage system and residue management on grain yield and nitrogen use efficiency in winter wheat in a cool Atlantic climate. Eur. J. Agron. 54, 61–69. doi:10.1016/j.eja.2013.11.009

Bünemann, E.K., Condron, L.M., 2007. Phosphorus and Sulphur Cycling in Terrestrial Ecosystems, in: Marschner, D.P., Rengel, P.D.Z. (Eds.), Nutrient Cycling in Terrestrial Ecosystems, Soil Biology. Springer Berlin Heidelberg, pp. 65–92.

Cardenas, E., Tiedje, J.M., 2008. New tools for discovering and characterizing microbial diversity. Curr. Opin. Biotechnol. 19, 544–9. doi:10.1016/j.copbio.2008.10.010

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. 8, 559–568.

Ceja-Navarro, J.A., Rivera-Orduña, F.N., Patiño-Zúñiga, L., Vila-Sanjurjo, A., Crossa, J., Govaerts, B., Dendooven, L., 2010. Phylogenetic and Multivariate Analyses To Determine the Effects of Different Tillage and Residue Management Practices on Soil Bacterial Communities. Appl. Environ. Microbiol. 76, 3685–3691. doi:10.1128/AEM.02726-09

Chan, K.Y., 2001. An overview of some tillage impacts on earthworm population abundance and diversity — implications for functioning in soils. Soil Tillage Res. 57, 179–191. doi:10.1016/S0167-1987(00)00173-2

Chen, B., Liu, E., Tian, Q., Yan, C., Zhang, Y., 2014. Soil nitrogen dynamics and crop residues. A review. Agron. Sustain. Dev. 34, 429–442. doi:10.1007/s13593-014-0207-8

Christopher, S.F., Lal, R., 2007. Nitrogen management affects carbon sequestration in North American cropland soils. Crit. Rev. Plant Sci. 26, 45–64. doi:10.1080/07352680601174830

Coppens, F., Garnier, P., De Gryze, S., Merckx, R., Recous, S., 2006. Soil moisture, carbon and nitrogen dynamics following incorporation and surface application of labelled crop residues in soil columns. Eur. J. Soil Sci. 57, 894–905. doi:10.1111/j.1365-2389.2006.00783.x

Cordell, D., Drangert, J.-O., White, S., 2009. The story of phosphorus: Global food security and food for thought. Glob. Environ. Change, Traditional Peoples and Climate Change 19, 292–305. doi:10.1016/j.gloenvcha.2008.10.009

Curry, J.P., Schmidt, O., 2007. The feeding ecology of earthworms–a review. Pedobiologia 50, 463–477.

De Oliveira, T., Bertrand, M., Roger-Estrade, J., 2012. Short-term effects of ploughing on the abundance and dynamics of two endogeic earthworm species in organic cropping systems in northern France. Soil Tillage Res. 119, 76–84.

Degrune, F., Dufrêne, M., Colinet, G., Massart, S., Taminiau, B., Bodson, B., Hiel, M.-P., Daube, G., Nezer, C., Vandenbol, M., 2015. A novel sub-phylum method discriminates better the impact of crop management on soil microbial community. Agron. Sustain. Dev. 1–10.

Deubel, A., Hofmann, B., Orzessek, D., 2011. Long-term effects of tillage on stratification and plant availability of phosphate and potassium in a loess chernozem. Soil Tillage Res. 117, 85–92. doi:10.1016/j.still.2011.09.001

Dolan, M.S., Clapp, C.E., Allmaras, R.R., Baker, J.M., Molina, J.A.E., 2006. Soil organic carbon and nitrogen in a Minnesota soil as related to tillage, residue and nitrogen management. Soil Tillage Res. 89, 221–231. doi:10.1016/j.still.2005.07.015

Eriksen-Hamel, N.S., Speratti, A.B., Whalen, J.K., Legere, A., Madramootoo, C.A., 2009. Earthworm populations and growth rates related to long-term crop residue and tillage management. Soil Tillage Res. 104, 311–316.

Fierer, 2003. Variations in microbial community composition through two soil depth profiles.

Fortune, T., Kennedy, T., Mitchell, B., Dunne, B., Murphy, K., Connery, J., Grace, J., 2005. Reduced cultivations - Update from Oak park experiments. Presented at the National Tillage Conference 2005.

Franzluebbers, A.J., 2002. Soil organic matter stratification ratio as an indicator of soil quality. Soil Tillage Res., Conservation Tillage and Stratification of Soil Properties 66, 95–106. doi:10.1016/S0167-1987(02)00018-1

Harrison, A.F., 1987. Soil organic phosphorus : a review of world literature | Clc. Wallingford : C.A.B. International.

Helgason, B.L., Walley, F.L., Germida, J.J., 2010. Long-term no-till management affects microbial biomass but not community composition in Canadian prairie agroecosytems. Soil Biol. Biochem. 42, 2192–2202. doi:10.1016/j.soilbio.2010.08.015

Helgason, B.L., Walley, F.L., Germida, J.J., 2009. Fungal and bacterial abundance in long-term no-till and intensive-till soils of the Northern Great Plains. Soil Sci. Soc. Am. J. 73, 120–127. doi:10.2136/sssaj2007.0392

Höflich, G., Tauschke, M., Kühn, G., Werner, K., Frielinghaus, M., Höhn, W., 1999. Influence of long-term conservation tillage on soil and rhizosphere microorganisms. Biol. Fertil. Soils 29, 81–86. doi:10.1007/s003740050528

Holland, J.M., 2004. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. Agric. Ecosyst. Environ. 103, 1–25.

Johnson-Maynard, J.L., Umiker, K.J., Guy, S.O., 2007. Earthworm dynamics and soil physical properties in the first three years of no-till management. Soil Tillage Res. 94, 338–345. doi:10.1016/j.still.2006.08.011

Kallenbach, C., Grandy, A.S., 2011. Controls over soil microbial biomass responses to carbon amendments in agricultural systems: A meta-analysis. Agric. Ecosyst. Environ. 144, 241–252. doi:10.1016/j.agee.2011.08.020

Kladivko, E.J., 2001. Tillage systems and soil ecology. Soil Tillage Res. 61, 61–76. doi:10.1016/S0167-1987(01)00179-9

Lemtiri, A., Colinet, G., Alabi, T., Cluzeau, D., Zirbes, L., Haubruge, E., Francis, F., 2014. Impacts of earthworms on soil components and dynamics. A review. Biotechnol. Agron. Soc. Environ. 18, 121–133.

Malhi, S.S., Lemke, R., Wang, Z.H., Chhabra, B.S., 2006. Tillage, nitrogen and crop residue effects on crop yield, nutrient uptake, soil quality, and greenhouse gas emissions. Soil Tillage Res. 90, 171–183. doi:10.1016/j.still.2005.09.001

Malhi, S.S., Nyborg, M., Goddard, T., Puurveen, D., 2010. Long-term tillage, straw and N rate effects on quantity and quality of organic C and N in a Gray Luvisol soil. Nutr. Cycl. Agroecosystems 90, 1–20. doi:10.1007/s10705-010-9399-8

McNeill, A., Unkovich, M., 2007. The Nitrogen Cycle in Terrestrial Ecosystems, in: Marschner, D.P., Rengel, P.D.Z. (Eds.), Nutrient Cycling in Terrestrial Ecosystems, Soil Biology. Springer Berlin Heidelberg, pp. 37–64.

Morris, N.L., Miller, P.C.H., Orson, J.H., Froud-Williams, R.J., 2010. The adoption of non-inversion tillage systems in the United Kingdom and the agronomic impact on soil, crops and the environment-A review. Soil Tillage Res. 108, 1–15. doi:10.1016/j.still.2010.03.004

Navarro-Noya, Y.E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M.C., Valenzuela-Encinas, C., Jiménez-Bueno, N., Verhulst, N., Govaerts, B., Dendooven, L., 2013. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biol. Biochem. 65, 86–95. doi:10.1016/j.soilbio.2013.05.009

Noack, S.R., McLaughlin, M.J., Smernik, R.J., McBeath, T.M., Armstrong, R.D., 2012. Crop residue phosphorus: speciation and potential bio-availability. Plant Soil 359, 375–385. doi:10.1007/s11104-012-1216-5

Nuutinen, V., 1992. Earthworm community response to tillage and residue management on different soil types in southern Finland. Soil Tillage Res. 23, 221–239.

Ohno, T., Erich, M.S., 1997. Inhibitory effects of crop residue-derived organic ligands on phosphate adsorption kinetics. J. Environ. Qual. 26, 889–895.

Palm, C.A., Myers, R.J., Nandwa, S.M., 1997. Combined use of organic and inorganic nutrient sources for soil fertility maintenance and replenishment. Replenishing Soil Fertil. Afr. 193–217.

Paul, E.A., 2014. Soil Microbiology, Ecology and Biochemistry. Academic Press.

Peigné, J., Cannavaciuolo, M., Gautronneau, Y., Aveline, A., Giteau, J.-L., Cluzeau, D., 2009. Earthworm populations under different tillage systems in organic farming. Soil Tillage Res. 104, 207–214.

Piegholdt, C., Geisseler, D., Koch, H.-J., Ludwig, B., 2013. Long-term tillage effects on the distribution of phosphorus fractions of loess soils in Germany. J. Plant Nutr. Soil Sci. 176, 217–226. doi:10.1002/jpln.201200393

Renneson, M., Dufey, J., Legrain, X., Genot, V., Bock, L., Colinet, G., 2013. Relationships between the P status of surface and deep horizons of agricultural soils under various cropping systems and for different soil types: A case study in Belgium. Soil Use Manag. 29, 94–102. doi:10.1111/j.1475-2743.2012.00435.x

Roesch, L.F.W., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., Daroub, S.H., Camargo, F.A.O., Farmerie, W.G., Triplett, E.W., 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J. 1, 283–290. doi:10.1038/ismej.2007.53

Rosas-Medina, M.Á., de León-González, F., Flores-Macías, A., Payán-Zelaya, F., Borderas-Tordesillas, F., Gutiérrez-Rodríguez, F., Fragoso-González, C., 2010. Effect of tillage, sampling date and soil depth on earthworm population on maize monoculture with continuous stover restitutions. Soil Tillage Res. 108, 37–42.

Schmidt, O., Clements, R.O., Donaldson, G., 2003. Why do cereal–legume intercrops support large earthworm populations? Appl. Soil Ecol. 22, 181–190. doi:10.1016/S0929-1393(02)00131-2

Sharpley, A.N., 2003. Soil mixing to decrease surface stratification of phosphorus in manured soils. J. Environ. Qual. 32, 1375–1384.

Shi, Y., Lalande, R., Hamel, C., Ziadi, N., Gagnon, B., Hu, Z., 2013. Seasonal variation of microbial biomass, activity, and community structure in soil under different tillage and phosphorus management practices. Biol. Fertil. Soils 49, 803–818. doi:10.1007/s00374-013-0773-y

Shuster, W.D., Shipitalo, M.J., Bohlen, P.J., Subler, S., Edwards, C.A., 2003. Population dynamics of ambient and altered earthworm communities in row-crop agroecosystems in the Midwestern U.S.: The 7th international symposium on earthworm ecology · Cardiff · Wales · 2002. Pedobiologia 47, 825–829. doi:10.1078/0031-4056-00266

Simonsen, J., Posner, J., Rosemeyer, M., Baldock, J., 2010. Endogeic and anecic earthworm abundance in six Midwestern cropping systems. Appl. Soil Ecol. 44, 147–155. doi:10.1016/j.apsoil.2009.11.005

Singh, J.S., Pandey, V.C., Singh, D.P., 2011. Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. Agric. Ecosyst. Environ. 140, 339–353. doi:10.1016/j.agee.2011.01.017

van Capelle, C., Schrader, S., Brunotte, J., 2012. Tillage-induced changes in the functional diversity of soil biota – A review with a focus on German data. Eur. J. Soil Biol. 50, 165–181. doi:10.1016/j.ejsobi.2012.02.005

van Groenigen, K.-J., Bloem, J., Bååth, E., Boeckx, P., Rousk, J., Bodé, S., Forristal, D., Jones, M.B., 2010. Abundance, production and stabilization of microbial biomass under conventional and reduced tillage. Soil Biol. Biochem. 42, 48–55. doi:10.1016/j.soilbio.2009.09.023

Varinderpal-Singh, Dhillon, N.S., Brar, B.S., 2006. Effect of incorporation of crop residues and organic manures on adsorption/desorption and bio-availability of phosphate. Nutr. Cycl. Agroecosystems 76, 95–108. doi:10.1007/s10705-006-9043-9

Vitousek, P.M., 2004. Nutrient cycling and limitation: Hawai ‘i as a model system Princeton University Press. Princet. N. J. USA.

Wang, J.B., Chen, Z.H., Chen, L.J., Zhu, A.N., Wu, Z.J., 2011. Surface soil phosphorus and phosphatase activities affected by tillage and crop residue input amounts. Plant Soil Environ. 57, 251–257.

Wang, J.J., Li, X.Y., Zhu, A.N., Zhang, X.K., Zhang, H.W., Liang, W.J., 2012. Effects of tillage and residue management on soil microbial communities in north china. Plant Soil Environ. 58, 28–33.

Wardle, D.A., 1995. Impacts of disturbance on detritus food webs in agro-ecosystems of contrasting tillage and weed management practices. Adv. Ecol. Res. 26, 105–185.

Wyss, E., Glasstetter, M., 1992. Tillage treatments and earthworm distribution in a Swiss experimental corn field. Soil Biol. Biochem. 24, 1635–1639.

Zaller, J.G., Köpke, U., 2004. Effects of traditional and biodynamic farmyard manure amendment on yields, soil chemical, biochemical and biological properties in a long-term field experiment. Biol. Fertil. Soils 40, 222–229. doi:10.1007/s00374-004-0772-0

Chapter III. A novel sub-phylum method

A novel sub-phylum method discriminates better the impact of crop management on soil microbial community

Published in *Agronomy for Sustainable Development*, 2015, 35 (3): 1157-1166.

Florine Degrune, Marc Dufrêne, Gilles Colinet, Sébastien Massart, Bernard Taminiau, Bernard Bodson, Marie-Pierre Hiel, Georges Daube, Carine Nezer, Micheline Vandenbol.

Outline

The aim of this publication was to explore the response of soil microbial diversity to different tillage regimes (conventional and reduced tillage) and crop residue management practices (residue retention and removal). In addition, we propose an original sub-phylum method for better distinguishing the impact of soil management on soil microbial diversity.

1. Abstract

Soil microorganisms such as mycorrhizae and plant-growth-promoting rhizobacteria have beneficial effects on crop productivity. Agricultural practices are known to impact soil microbial communities, but past studies examining this impact have focused mostly on one or two taxonomic levels, such as phylum and class, thus missing potentially relevant information from lower levels. Therefore we propose here an original, sub-phylum method for studying how agricultural practices modify microbial communities. This method involves exploiting the available sequence information at the lowest taxonomic level attainable for each operational taxonomic unit. In order to validate this novel method we assessed microbial community composition using 454 pyrosequencing of 16S and 28S rRNA genes, then we compared the results with results of a phylum-level analysis. Agricultural practices included conventional tillage, reduced tillage, residue removal and residue retention. Results show that, at the lowest taxonomic level attainable, tillage is the main factor influencing both bacterial community composition, accounting for 13% of the variation, and fungal community composition, accounting for 18% of the variation. Whereas phylum-level analysis failed to reveal any effect of soil practice on bacterial community composition, and missed the fact that different members of the same phylum responded differently to tillage practice. For instance, the fungal phylum Chytridiomycota showed no impact of soil treatment, while sub-phylum-level analysis revealed an impact of tillage practice on the Chytridiomycota sub-groups *Gibberella*, which includes a notorious wheat pathogen, and Trichocomaceae. This clearly demonstrates the necessity of exploiting the information obtainable at sub-phylum level when assessing the effects of agricultural practice on microbial communities.

Keywords

Microbial diversity, microbial community composition, taxonomical level, pyrosequencing, conservation agriculture

2. Introduction

Soil microorganisms are abundant and diverse and can have both beneficial and adverse effects on crop growth. Some, such as plant-growth-promoting rhizobacteria and mycorrhizae, are well known to favour crop productivity and plant health (Berg, 2009; Siddiqui et al., 2008). They are notably involved in key processes such as improving plant nutrient acquisition, and they also play a major role in stimulating plant growth and in protecting plants against pathogens by producing bioactive substances. Conversely, agricultural practices influence the physical and chemical properties of the soil and hence affect the abundance and diversity of soil microorganisms (Kladivko 2001; Helgason et al. 2009; Lienhard et al. 2013). This generates interest in studying the responses of microbial communities to agricultural practices.

Powerful new tools are now available for assessing at very high resolution the huge diversity of microbial communities and the composition thereof. One is massive DNA (pyro)sequencing, which generates thousands of DNA sequences (Cardenas and Tiedje 2008) in record time. In addition, the recent introduction of multivariate analysis in microbial ecology has made it possible to summarize and explore such data, to detect microbial patterns and relate them to the environment (Ramette 2007). A central question in such studies remains: how to choose the taxonomic level used to detect microbial patterns?

The most recent surveys based on massive DNA sequencing and multivariate analysis and aiming to detect microbial patterns in an agricultural context have focused on a high taxonomic level, i.e. class or phylum (Lienhard et al. 2013; Ceja-Navarro et al. 2010; Navarro-Noya et al. 2013). Such studies allow a coarse assessment of the variability of large microbial groups in relation to agricultural practices. This approach, however, ignores a large part of the accessible information concerning lower taxonomic levels, which could be more relevant to agriculture. For example, Ascomycota is a vast group of fungi containing both beneficial and harmful organisms, the latter being illustrated by the genus Gibberella, which includes the causative agent of Fusarium head blight of wheat (Bottalico and Perrone 2002). In addition, a phylum or class can contain subgroups of organisms responding differently to environmental factors. For example, subgroups of Acidobacteria, one of the most abundant bacterial phyla in many soils, are reported to respond differently to tillage practice (Yin et al. 2010).

On the other hand, detecting microbial patterns at a finer taxonomic level such as genus or species remains difficult or even unfeasible, because a great many soil microbes remain unknown at these levels, and because pyrosequenced DNA fragments are still too short to allow accurately assigning the sequence at these levels.

Consequently, we propose an original method to increase the resolution of the analysis by exploiting a maximum of information in the dataset, a method that could provide better discrimination between microbial communities according to the agricultural soil practice. The method is to exploit the available sequence information at the lowest taxonomic level attainable for each operational taxonomic unit.

To test the usefulness of this method, we have used it to examine the effects of tillage and crop residue management practice (**Figure 15**) on microbial community composition, and have compared the results obtained with those of a phylum-level analysis of the same soil samples. For this we have used 16S and 28S pyrosequencing followed by redundancy ordination analysis.

Figure 15 Different soil treatments applied to the experimental field: a reduced tillage, crop residues being left at the soil surface; b conventional tillage, the cover crop having been mixed into the soil by plowing. Both pictures show the appearance of the soil before and after passage of the machine which prepares the soil and sows simultaneously.

3. Materials and Methods

Site description

The studied site is located on the experimental farm of Gembloux Agro-Bio Tech (University of Liège, Gembloux, Belgium, at 50°33'45.92"N and 4°42'48.97"E). According to the WRB soil system, the soil type of the studied site is classified as Cutanic Luvisol. The soil texture is silt loam (FAO) with 18-22% clay, 70-80% silt, and 5-10% sand particles, and the organic matter is characterized by a C:N ratio between 10 and 12. The Belgian climate is maritime temperate, with cool, humid summers and mild, rainy winters. The monthly average temperature is highest in July, at 18.4°C, and lowest in January, at 3.3°C. The monthly average rainfall is highest in December, at 81 mm, and lowest in April, at 51.3 mm (data from the Belgian Royal Meteorological Institute).

Soil treatments and experimental design

The experimental design consisted of a Latin square arrangement with four replicates of four soil treatments. Each soil treatment consisted of a combination of different soil practices: a tillage practice (conventional or reduced tillage) with a crop residue management practice (residue retention or removal). The combinations were as follows: conventional tillage with residue removal (CT/-R, the agricultural practice most commonly used in Belgium), conventional tillage with residue retention (CT/+R), reduced tillage with residue retention (RT/+R), and reduced tillage with residue removal (RT/-R). Conventional tilled plots were ploughed to a depth of 25 cm, while in reduced-tillage plots only the top 10 cm of soil was mixed. The estimated quantity of crop residues from the 2012 season was 8.3 tons/ha for the plots with residue retention (+R) and 4.5 tons/ha for the plots with residue removal (-R). Crops are rotated on the studied field, and the experimental design and different soil treatments have been applied since autumn, 2008. Crop history is as follows: *Brassica napus* (2009), *Triticum aestivum* (2010, 2011, and 2012), and *Vicia faba* (2013).

Soil sampling and physicochemical analyses

We took sixteen soil samples from the faba bean field in April 2013, 10 days after sowing and one month after glyphosate application. Each sample was a composite of five 25-g subsamples. Each subsample consisted of a 5-cm core collected corresponding to a depth of 15 to 20 cm. This depth was chosen to allow comparisons with other studies conducted by our laboratory and because we wanted to focus on the soil layer located between the depth reached by reduced tillage

(7 cm) and that reached by conventional tillage (25 cm). One should note that the response of microbial communities is related to crop residue location, which is different for conventional and reduced tillage (Helgason et al. 2014). Under conventional tillage, residues are mixed within the soil profile, while under reduced tillage, there is a stratification of residues. For each sample, we performed physical and chemical soil analyses. Volumetric water content and porosity were measured by the normalized cylinder method (AFNOR NF X31-501). Clay content was measured by the normalized pipette method (AFNOR NF X31-107). Soil nitrates were determined by the QuickChem®: method 12-107-04-1-B. Soil pH was measured in 1 N KCL (2:5 w:v). Water-extractable elements were quantified by flame absorption (Ca, Mg), flame emission (P, Na), or colorimetry (P) after extraction of 20 g of 8-mm-sieved fresh soil in 100 ml H2O for 1 h at room temperature and filtration on 602 H 1/2. Carbon was quantified as described by Ghani et al. (2003). The average soil physicochemical parameters characterizing each treatment (CT/R+, CT/R-, RT/R+, and RT/R-) are presented in **Table 3**. We performed a statistical test (ANOVA, n=4) to assess the impacts of tillage practice and crop residue management on the log-transformed soil parameters. The results (**Table 3**) show a variation in potassium, phosphorus, sodium, porosity, pH, and nitrates between conventional and reduced tillage, all these values being higher under conventional tillage. There was no impact of residue management (residue retention or removal) on soil parameters.

DNA extraction and pyrosequencing of 16S and 28S rRNA gene sequences

We used the PowerMax® soil DNA isolation kit (MO BIO Laboratories, Solana Beach, CA) to extract metagenomic DNA from 8 grams (wet weight) of each composite sample, according to the manufacturer's recommendations. We checked the quality of the DNA by gel electrophoresis and we quantified it with the Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) prior to storage at -20°C. We used Roche 454-pyrosequencing technology to sequence fragments of the 16S and 28S rRNA genes. For bacterial DNA the procedure was briefly as follows: we carried out a PCR to amplify a 500-bp fragment of the 16S rRNA gene from the total bacterial DNA. We used primers designed by fusion of (1) primers targeting the 16S rRNA gene fragments E9-29: 5'-GAGAGTTTGATCATGGCTCAG-3' and E530-541: 5'-ACCGCGGCTGCTGGCAC-3' (Baker et al. 2003), (2) MIDs (multiplex identifiers), and (3) the Roche 454 pyrosequencing adaptors (Roche Diagnostics, Vilvoorde, Belgium). Our PCR method could be biased, as we directly amplified our target using a fusion primer (Berry et al. 2011). However, the bias is the same for each sample we studied. We performed the PCR under the following conditions: the amplification mix contained 5 U FastStart

High Fidelity DNA polymerase (Roche Diagnostics, Vilvoorde, Belgium), 1x enzyme reaction buffer, 200 µM dNTPs (Eurogentec, Liège, Belgium), each primer at 0.2 µM, and 100 ng genomic DNA in a final volume of 100 µl. Thermocycling conditions were: denaturation at 94 °C for 15 min followed by 25 cycles of 94 °C for 40 s, 56 °C for 40 s, 72 °C for 1 min, and a final 7-min elongation step at 72 °C. We carried out amplification on a Mastercycler ep Gradient thermocycler (Eppendorf, Hamburg, Germany). PCR products were electrophoresed through a 1% agarose gel and the DNA fragments were plugged out and purified with the SV PCR Purification Kit (Promega Benelux, Leiden, the Netherlands). We assessed the quality and quantity of the products with a Picogreen dsDNA quantitation assay (Isogen, St-Pieters-Leeuw, Belgium). We sequenced all amplicons with the Roche GS-Junior Genome Sequencer (Roche, Vilvoorde, Belgium). For fungi the procedure was the same, except that we amplified and sequenced a 500-pb fragment of the 28S rRNA gene with the following primers: NL-1; 5'-GCATATCAATAAGCGGAGGAAAAG–3' and NL-4; 5'-GGTCCGTGTTTCAAGACGG-3' (Kurtzman and Robnett 1997).

Table 3 Average physicochemical soil parameters according to the soil treatment (tillage practice: conventional or reduced tillage) and type of crop residue management (residue retention or residue removal).

Physic and chemical soil parameters	Unit	Conventional tillage – Residue retention	Conventional tillage – Residue removal	Reduced tillage – Residue retention	Reduced tillage – Residue removal
Texture					
Sand	%	5.7±0.8	5.9±0.9	5.9±0.5	5.5±0.6
Silt	%	78.2±2.3	78.0±1.6	78.1±1.4	78.9±1.6
Clay	%	16.2±2.1	16.2±2.3	16.1±1.3	15.6±1.7
Water-extractable elements					
Carbon	mg/kg 105°C	368.3±53	350.2±52	373.1±57	385.0±60
Calcium		45.4±10.2	38.8±3.4	44.8±13.5	42.8±11.4
Potassium *Tillage***		17.1±3.8 **(a)**	13.7±4.2 **(a)**	11.3±4.3 **(b)**	8.6±3.6 **(b)**
Phosphorus *Tillage**		3.9±1.0 **(a)**	3.9±1.1 **(a)**	3.4±1.4 **(b)**	2.7±0.9 **(b)**
Sodium *Tillage**		21.2±0.9 **(a)**	20.6±0.4 **(a)**	20.3±1.1 **(b)**	19.6±0.8 **(b)**
Magnesium		2.7±0.5	2.6±0.3	2.9±0.7	2.7±0.6
Porosity *Tillage***	%	46.9±1.7 **(a)**	45.8±2.0 **(a)**	43.3±1.4 **(b)**	43.0±2.9 **(b)**
Water content	%	33.4±0.3	32.4±1.0	32.2±1.0	32.3±0.5
pH *Tillage**	-	6.6±0.1 **(a)**	6.6±0.2 **(a)**	6.4±0.2 **(b)**	6.3±0.1 **(b)**
Nitrates *Tillage***	kg/ha	13.3±2.4 **(a)**	15.5±5.0 **(a)**	7.3±2.2 **(b)**	6.9±4.8 **(b)**
Total organic carbon	%	1.1±0.1	1.1±0.1	1.2±0.1	1.1±0.1

A statistical test (ANOVA, n=4) was performed to assess the impact of soil treatment on log-transformed soil parameters. Lines in bold with letters mean there is an effect of soil treatment. Different letters correspond to significantly different values. The significance level is as follows: * significant at the 0.05 probability level and ** significant at the 0.01 probability level.

Bioinformatics analysis of the pyrosequencing data

A total of 85935 raw reads were obtained for bacteria and 82119 for fungi. The obtained partial 16S and 28S rRNA gene sequences were processed with the MOTHUR package (Schloss et al. 2009). We denoised all sequence reads with the Pyronoise algorithm implemented in MOTHUR and filtered them according to the following criteria: minimal length: 425 bp; an exact match to the barcode and 1 mismatch allowed for the proximal primer. We used ChimeraSlayer to check the sequences for the presence of chimeric amplifications (Haas et al. 2011). The numbers of high-quality reads obtained after read processing were 68230 for bacteria and 66337 for fungi. We compared the resulting high-quality read sets with a reference dataset of aligned sequences of the corresponding region derived from the SILVA 111 database of full-length rDNA sequences implemented in MOTHUR. To cluster the final reads into operational taxonomic units, we used in MOTHUR the nearest neighbor algorithm with a 0.03 distance unit cutoff. A taxonomic identity was attributed to each operational taxonomic unit by comparison with the SILVA database (80% homogeneity cutoff). The raw data sets are available in the SRA database (Sequence Read Archive) under project accession number SRP043491 for bacteria and under project accession number SRP044036 for fungi.

Statistical analyses

We performed all statistical analyses with R statistical software (Team 2013). We analysed the impact of tillage practice and crop residue management on microbial alpha diversity and microbial community composition. This analysis was done at two taxonomic levels: phylum level and the most precise taxonomic level that could be reached for each operational taxonomic unit.

Microbial alpha diversity analysis

As using samples with different sequencing depths can bias alpha diversity indexes, a random sequence subsampling step was carried out so as to compare samples containing the same number of sequences: that of the sample having the lowest sampling depth (2693 sequences for bacteria and 2453 for fungi). To measure the alpha diversity of bacteria and fungi in the different subsamples, we used MOTHUR to evaluate the richness and Shannon indexes. We performed an ANOVA (n=4) to determine if the alpha diversity changed with the soil treatment applied. We used the operational taxonomic unit level to measure alpha diversity.

Microbial community composition analysis

We used multivariate analysis to relate microbial community composition to soil treatment, i.e. tillage practice (conventional or reduced tillage) and type of crop residue management (residue retention or removal). We determined the impact of soil treatment on bacterial and fungal community composition at two taxonomic levels: phylum level and the most precise taxonomic level possible for each operational taxonomic unit, called the 'precise level' in the following text. For the analysis, microbial abundance data was first log2 transformed with the decostand() function implemented in the vegan package (Oksanen et al. 2007). The log2 transformation was chosen to weight the variation of dominant taxa abundance in a reasonable way. We used redundancy ordination analysis to analyze and compare relationships between microbial community composition at each taxonomic level and soil practice. We constrained the redundancy ordination analysis by 3 explanatory variables: tillage practice, crop residue management practice, and the interaction of both. We used the ordistep() function of vegan to select the most significant explanatory variable. In addition, we used the envfit() function of vegan to fit soil physical and chemical parameters to the ordination graph, as these parameters, related to soil practices, might explain microbial composition variability. Finally, we revealed the bacteria and fungi most strongly affected by the best explanatory variable with the goodness() function of vegan.

4. Results and discussion

With a view to achieving better discrimination power than is usual in such studies, we compared two methods of microbial community composition analysis applied to soils subjected to different tillage practices: conventional and reduced tillage, and different residue management practices: crop residue retention and removal. One approach was to limit our analysis to the phylum level (the level most studied in soil microbial surveys), the second being to use the most precise taxonomic level reachable for each operational taxonomic unit. We then assessed the information gain provided by the more precise analysis.

Phylum composition of microbial communities

Our analyses showed that, for each soil treatment, Proteobacteria (25%-29%), Acidobacteria (18%-24%), and Bacteroidetes (9%-14%) were the most abundant bacterial phyla (**Figure 16a**). These phyla are often dominant in very diverse agricultural soils (Janssen 2006; Lienhard et al. 2013; Navarro-Noya et al. 2013). We also showed that the most abundant fungal phyla for each combination were Ascomycota (74%-86%) and Basidiomycota (12%-25%), which are saprotrophic soil fungi (de Boer et al. 2005) frequently dominant in soil ecosystems (Lienhard et al. 2013; Buée et al. 2009) (**Figure 16b**).

Analysis of alpha diversity in relation to soil treatment

The average soil alpha diversity characterizing each soil treatment and the numbers of sequences before and after the subsampling step are summarized in **Table 4**. We performed a statistical test (ANOVA, n=4) to assess the effect of soil practice (conventional or reduced tillage) and crop residue management practice (residue retention or removal) on the alpha diversity indexes.

Fungal richness appeared lower than bacterial richness (**Table 4**), with fewer than 300 operational taxonomic units for fungi and more than 1000 for bacteria. Strangely, this very low fungal richness is comparable to that observed in agricultural soils with high aluminium toxicity (Lienhard et al. 2013). In our soil, the low fungal richness might be due to cultivation history, as for a long time before 2008, the experimental field was subjected to conventional tillage with tilling tools liable to disturb fungal hyphae.

After only 4 years of experiment, we can already observe differences in bacterial and fungal alpha diversity indexes between conventional and reduced tillage. The diversity of bacteria appeared higher under conventional tillage (**Table 4**). This could be due to the physical disturbance caused by tillage. Aggregates are broken and the organic matter is released and available for bacterial activity (Cheeke et al. 2012). Our results are consistent with those of Lienhard et al. (2013) and Navarro-Noya et al. (2013), showing an increase in bacterial diversity with increased soil disturbance and cropping intensity. In addition, Siciliano et al. (2014) have shown soil fertility, including nitrates and organic matter, to be the most important factor influencing bacterial and fungal richness and diversity indexes. In our soil, the nitrate content was consistently higher under conventional tillage (**Table 3**), which could also explain the observed higher bacterial diversity under conventional tillage.

Fungal richness appeared higher under conventional tillage than under reduced tillage, and higher with residue removal than with residue retention. Our results differ from those of Lienhard et al. (2013), who observed a negative effect of tillage on fungal richness and suggested that this effect could be due to a negative effect of tilling tools on the growth of fungal hyphae. Yet as for bacterial diversity, the higher fungal richness observed under conventional tillage could be caused by the higher nitrate content observed under conventional tillage. Here, the higher nitrate content under conventional tillage seems to be a factor influencing fungal richness more strongly than the disturbance of fungal hyphae caused by tilling tools. Although the use of such indexes is an easy way for an ecologist to assess diversity, these indexes ignore taxonomic identity, treating operational taxonomic units as anonymous entities (Hartmann and Widmer 2006). It is therefore interesting to analyse further such complex soil microbial communities with a method such as ordination that takes microbial community composition into account. However, using such a method requires choosing an appropriate taxonomic level.

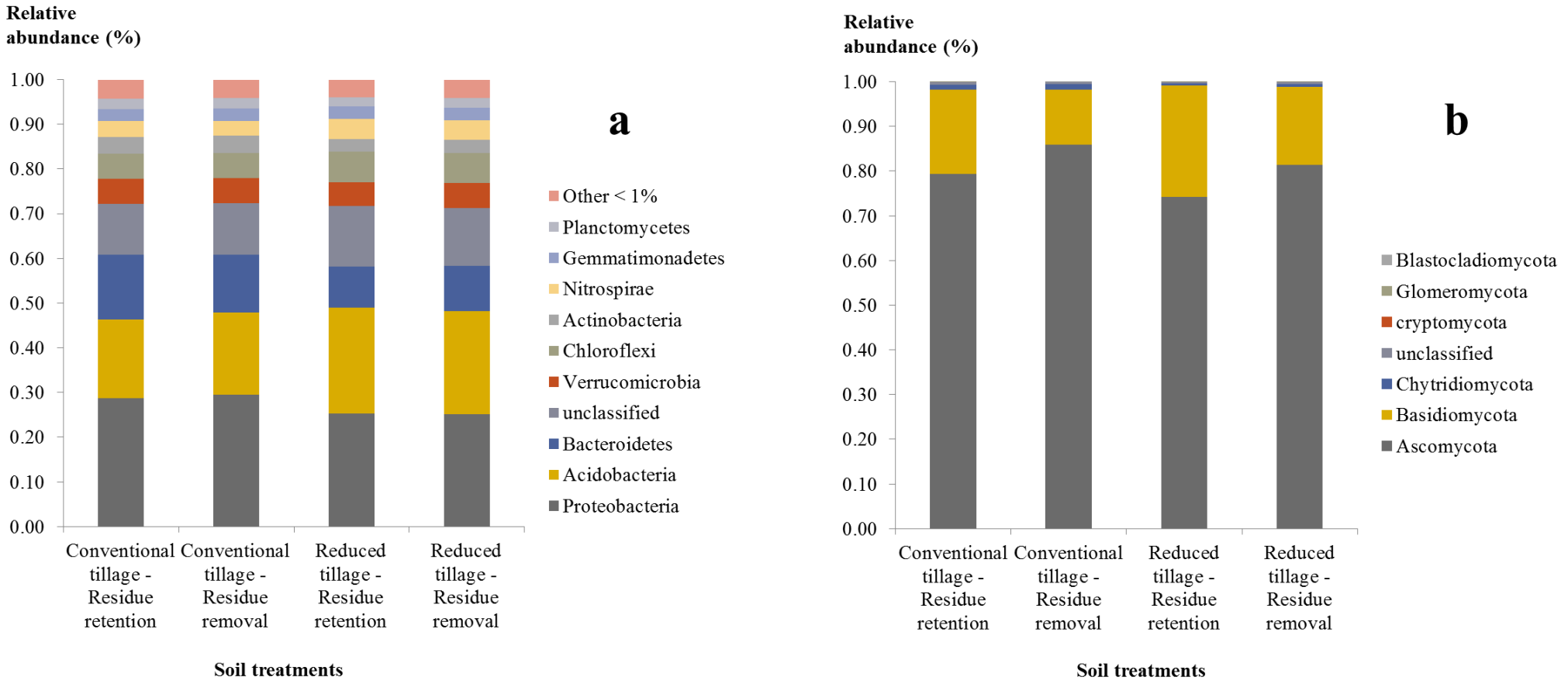


Figure 16 Barplot representation of the relative abundances for each treatment (based on the sums of the 4 replicates) of a soil bacterial and b soil fungal phyla.

Table 4 Soil alpha diversity indexes for each treatment, on the basis of operational taxonomic units (based on averages of 4 replicates).

	Conventional tillage – Residue retention	Conventional tillage – Residue removal	Reduced tillage – Residue retention	Reduced tillage – Residue removal
		Bacteria		
Number of reads before/after subsampling	4408/2693	4274/2693	3926/2693	4451/2693
Richness index	1776±313	1762±311	1413±357	1571±236
Shannon index Tillage***	6.62±0.02 **(a)**	6.63±0.03 **(a)**	6.37±0.10 **(b)**	6.38±0.13 **(b)**
		Fungi		
Number of reads before/after subsampling	3993/2453	4048/2453	4418/2453	4186/2453
Richness index Tillage** Residues*	251±24 **(b)**	291±40 **(a)**	227±11 **(d)**	235±13 **(c)**
Shannon index	3.82±0.38	3.88±0.12	3.80±0.17	3.83±0.06

We performed a statistical test (ANOVA, n=4) to assess the impact of soil management practice (tillage practice and crop residue management practice) on log-transformed indexes. Lines with letters mean there was an effect of treatment. Different letters correspond to significantly different values. The numbers of sequences before and after the subsampling step are also given. Significance values are as follows: * significant at the 0.05 probability level, ** significant at the 0.01 probability level and *** significant at the 0.001 probability level

Effect of soil practice on microbial community composition evaluated at two taxonomic levels

Bacterial community composition analysis

At phylum level (**Figure 17a**), we observed no effect of soil practice on bacterial community composition. We did not focus on cropping intensity, but it is worth noting that Lienhard et al. (2013) report clear phylum-level differences in bacterial community composition along a cropping intensity gradient. This suggests that changing the cropping intensity alters the soil conditions more drastically than do our changes in soil practice, making it possible to detect coarser (phylum-level) changes in bacterial communities. That our changes in soil practice are milder is supported by the low percentage of variance along the first two axes of our ordination plot (**Figure 17a**).

At the precise level, however (**Figure 17b**), we did observe a significant shift in bacterial composition according to the soil practice used. Tillage practice appeared as the best explanatory variable, explaining 13% of the variation in bacterial composition ($p<0.01$). Our results demonstrate that it is useful to exploit the information that can be obtained at sub-phylum level, particularly in a system with lesser contrast between soil treatments, since the effect of tillage practice was not detectable at phylum level. At the more precise level, we were able to obtain sub-phylum-level information on the bacteria impacted by tillage practice. For example, we showed that the relative abundances of bacteria of the groups Methylocystaceae, *Sphingomonas*, Saprospiraceae, Oxalobacteraceae, and Chitinophaga were higher under conventional tillage.

Some of the groups just mentioned could play key roles in crop health and growth. For example, Methylocystaceae (**Figure 17b**) is a group of methanotrophs, i. e. bacteria using methane (CH4) as energy source under aerobic conditions and thus capable of reducing methane emissions (Conrad 1996). Our results suggest that conventional tillage generates favorable conditions for Methylocystaceae development. The higher P and K contents observed (**Table 3**) under conventional tillage might explain our results, as Zheng et al. (2013) have found P and K amendments to increase the methanotroph population significantly. Interestingly, their survey evidenced a negative correlation between methanotrophic activity and methanotroph abundance. Species of the genus *Sphingomonas* (**Figure 17b**) are involved in degrading refractory contaminants such as herbicides (Sørensen et al. 2001). Our results suggest that conventional tillage favors such organisms. The application of glyphosate to our field one month before soil sampling might have

induced microbial glyphosate-degrading activity, which is higher under aerobic conditions (Rueppel et al. 1977). As we observed higher soil porosity under conventional tillage than under reduced tillage but similar water content regardless of the tillage practice (**Table 3**), we could expect a higher oxygen content under conventional tillage and hence better development of microorganisms capable of degrading glyphosate under aerobic conditions.

Fungal community composition analysis

At phylum level (**Figure 18a**), we observed a significant shift in fungal community composition according to the tillage practice ($p<0.05$). The shift was largely due to Chytridiomycota (C), favored under conventional tillage, and Basidiomycota, favored under reduced tillage. Fungi of the phylum Basidiomycota are known to degrade lignin and cellulose under anaerobic conditions (de Boer et al. 2005). Given the soil humidity, which was similar for conventional and reduced tillage, and the soil porosity, which was higher under reduced tillage (**Table 3**), we could expect such anaerobic conditions to be more frequent under reduced tillage. Little information is available on the diverse groups of fungi composing the Chytridiomycota, but soil Chytridiomycota appears capable of recovering from dryness and high temperature (Gleason et al. 2004), more likely to occur in tilled soil.

These same two phyla were likewise highlighted in the survey of Lienhard et al. (2013), showing a greater relative abundance of Chytridiomycota under high cropping intensity (conventional tillage) and a greater relative abundance of Basidiomycota under lower cropping intensity (zero tillage). It thus appears that the contrast between our soil treatments, insufficient to induce differences between bacterial communities detectable at phylum level, was sufficient to induce differences between fungal populations detectable at this level. This might be due to the lower diversity of fungi as compared to bacteria (**Table 4**). It is generally accepted that a population with a low diversity should be less stable under environmental stress, as species affected by the stress will not be replaced by others, as in the case of a more diverse population (Giller et al. 1997).

At the more precise level (**Figure 18b**), however, we observed a significant shift in fungal community composition according to the tillage practice, which explained 18% of the variation in community composition ($p<0.01$). The precise analysis showed that different members of the phylum Chytridiomycota (C) responded differently to tillage practice: the relative abundance of Chytridiomycetes (C) was higher under conventional tillage, but the relative abundance of Powellomyces (C) was higher under reduced tillage.

Some phyla, furthermore, showed no impact of soil management practice (**Figure 18a**), while analysis at the precise level (**Figure 18b**) revealed an effect of tillage on the relative abundance of certain phylum members. A difference was observed, for example, between two subgroups of the phylum Ascomycota: a higher relative abundance was observed for *Gibberella* (A) under conventional tillage and for Trichocomaceae (A) under reduced tillage. These results again highlight the importance of comparing communities of soil fungi at the most precise taxonomic level accessible.

Some of these taxa are known to have specific roles in ecosystems. For example, *Gibberella zeae*, also known as *Fusarium graminearum*, is the causative agent of Fusarium head blight of wheat (Bottalico 1998). This disease can cause root, stem, and ear decay, resulting in a significant reduction in crop yield. As reported by Booth (1971), *F. graminearum* can survive saprophytically on a wide range of gramineous host debris, such as wheat residues. As our samples were taken at a depth between 15 and 20 cm, the higher relative abundance of *F. graminearum* observed under conventional tillage might be due to the presence of crop residues from previous wheat crops at this depth, while crop residues remain in the topsoil (<10 cm) under reduced tillage.

For both bacteria and fungi, the observed pattern changes can be explained by differences in soil conditions between conventional and reduced tillage. We show here that several soil parameters, including porosity, potassium, nitrates, pH, sodium, and phosphorus, were higher under conventional tillage and might explain variations in bacterial community composition. Among these factors, the pH has been recognized as the best driver of changes in bacterial community composition and diversity, while fungal community composition appears closely associated with changes in nutrient status, such as phosphorus and the C:N ratio. (Lauber et al. 2008). Here we show a variation in nitrates and phosphorus between conventional and reduced tillage, which might explain the observed fungal pattern changes.

By exploiting the data obtainable at a more precise taxonomic level, we are able to go further in our analysis and to identify groups of organisms that are affected by soil management practice. For example, because *Gibberella zeae* has a negative effect on wheat, information on its higher relative abundance under conventional tillage is relevant to farmers, who can expect to see the disease under such soil practice. This information is missed when the data are analyzed at phylum level. However, to exploit the available data on microbial community composition, agronomists need to

know more about the roles played by soil microorganisms in their environment, and about their effects on plant health and growth. For many taxa, such information is still hard to obtain.

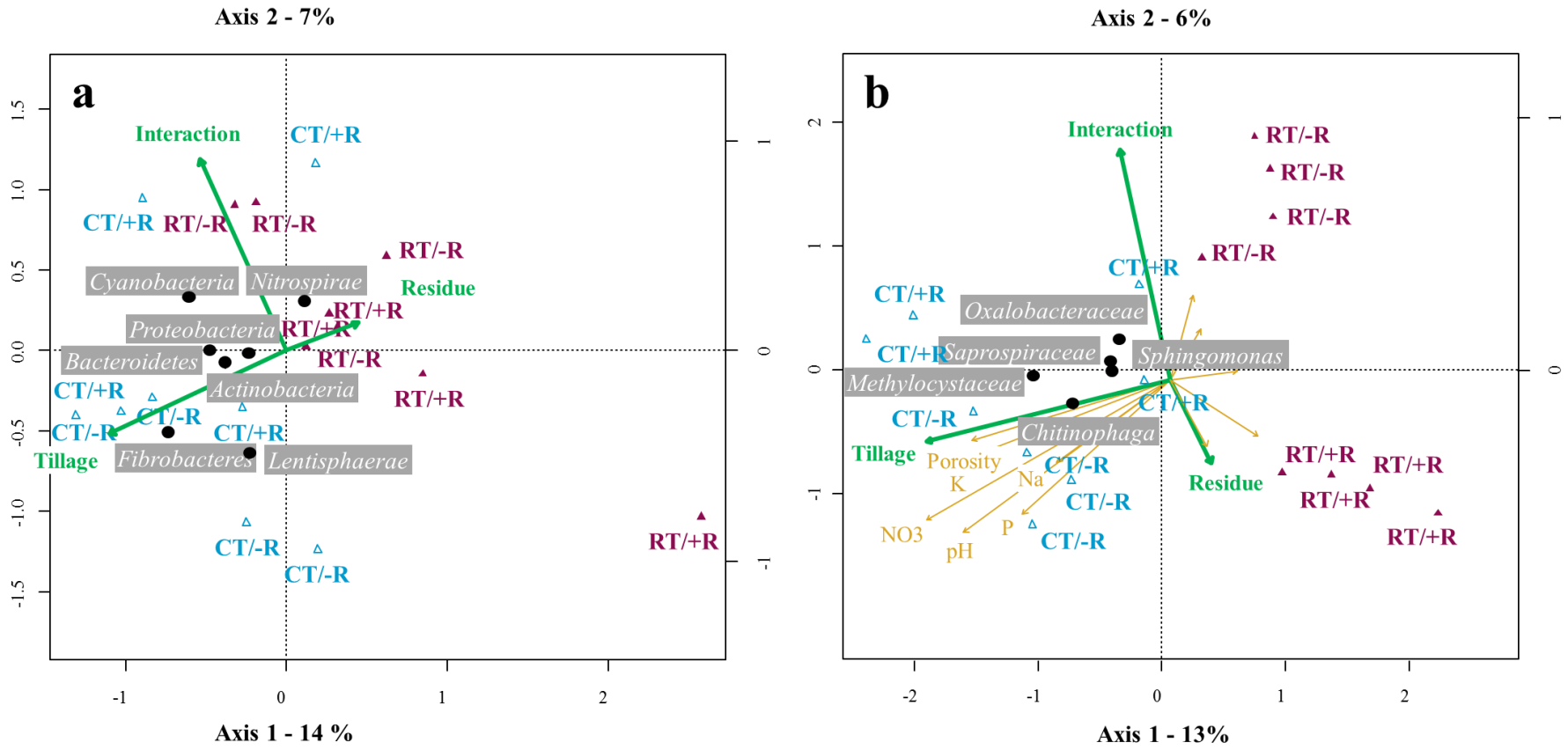


Figure 17 Factorial map of the redundancy analysis carried out on soil bacterial community composition at a phylum level and b the most precise taxonomic level attainable. Axes 1 and 2 represent the maximum percentage of variance that can be explained by soil practice: tillage practice (conventional tillage; CT or reduced tillage; RT) and crop residue management practice (residue retention; R+ or residue removal; R-). For both analyses, a statistical test (ANOVA, n=4) was performed to assess the effect of soil management practice on bacterial community composition. At phylum level, there appeared no difference in bacterial community between soil management practices, while differences due to tillage practice were observed at the more precise level, this factor accounting for 13% of the bacterial community between soil management practices, while differences due to tillage practice were observed at the more precise level, this factor accounting for 13% of the bacterial community variation ($p<0.01$).

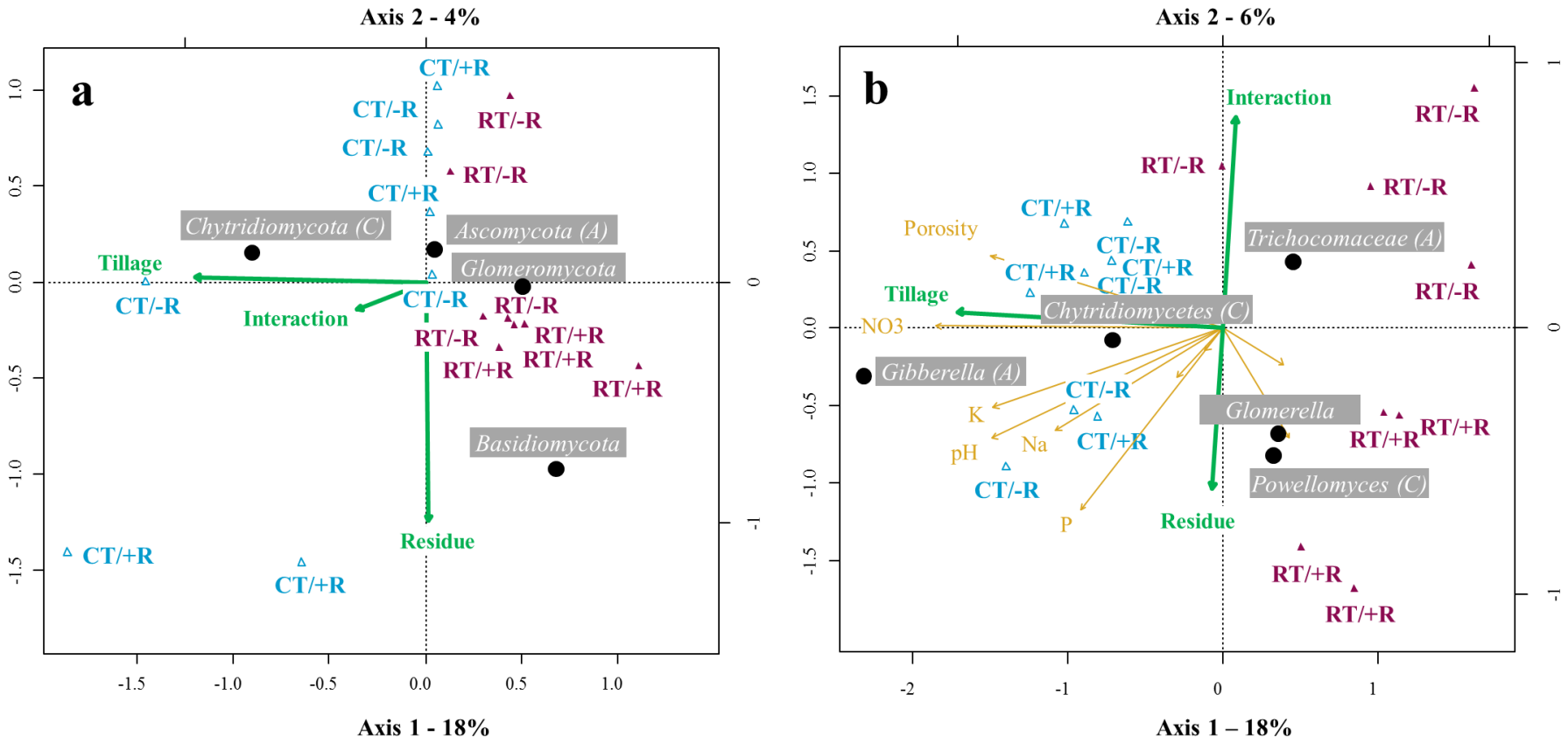


Figure 18 Factorial map of the redundancy ordination analysis of soil fungal community composition at a phylum level and b the most precise taxonomic level attainable. Axes 1 and 2 represent the maximum percentages of variance that can be explained by soil practice, i.e. by tillage practice (conventional tillage, CT; reduced tillage, RT) and crop residue management practice (residue retention, R+; residue removal, R-). For both analyses, a statistical test (ANOVA, n=4) was performed to assess the effect of soil practice on fungal community composition. At both phylum level and the more precise level, there appear differences in fungal community composition between soil practices, with tillage practice accounting, respectively, for 16% and 18% of the variation ($p<0.05$ and $p<0.01$).

5. Conclusion

In the present work we have attempted to improve the discrimination power of microbial community analysis applied to soils subjected to different tillage and residue management practices. For this we have assessed the importance of exploiting 16S and 28S rRNA gene sequencing data at sub-phylum level to identify effects of soil management practice. Our results highlight tillage practice as an important factor influencing microbial community composition. Plowing notably affects several physico-chemical parameters that contribute greatly to shaping the microbial habitat: soil porosity, pH, and the NO3, P, K, and Na contents. These can be expected to affect microbial community composition. Most importantly, we show that some effects of tillage observed at sub-phylum level escape notice at phylum level, and that some effects detectable at this higher taxonomic level mask differences in the responses of different members of a same phylum. Clearly, phylum-level analysis cannot do justice to the diversity of organisms within a phylum. As on the other hand it is currently impossible to assign a genus or species to each operational taxonomic unit, we recommend the compromise described in this paper: using for each operational taxonomic unit the most precise taxonomic level attainable. This method should facilitate a fine-scaled and detailed assessment of microbial communities across different soil practices.

Acknowledgments

We express our sincere gratitude to Gembloux Agro Bio-tech, University of Liège and to its staff for providing funding, infrastructure, and valuable time to support this project. This project is part of the AgricultureIsLife platform coordinated by Sarah Garré, and which is an initiative of Gembloux Agro Bio-tech to enhance global agricultural practices. We also want to thank the 'Unité Fertilité des Sols et Protection des Eaux' (CRAw) for nitrogen measurements.

6. References

Baker G, Smith J, Cowan DA (2003) Review and re-analysis of domain-specific 16S primers. J Microbiol Methods 55 (3):541-555. doi:10.1016/j.mimet.2003.08.009

Berg G (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84 (1):11-18. doi:10.1007/s00253-009-2092-7

Berry D, Mahfoudh KB, Wagner M, Loy A (2011) Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. Appl Environ Microbiol 77 (21):7846-7849. doi:10.1128/AEM.05220-11

Booth C (1971) The genus Fusarium. Commonwealth Mycological Institute, Kew, UK

Bottalico A (1998) Fusarium diseases of cereals: species complex and related mycotoxin profiles, in Europe. J Plant Pathol 80 (2):85-103

Bottalico A, Perrone G (2002) Toxigenic Fusarium species and Mycotoxins Associated with Head Blight in Small-Grain Cereals in Europe. Eur J Plant Pathol 108 (7):611-624. doi:10.1023/A:1020635214971

Buée M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F (2009) 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol 184 (2):449-456. doi:10.1111/j.1469-8137.2009.03003.x

Cardenas E, Tiedje JM (2008) New tools for discovering and characterizing microbial diversity. Curr Opin Biotechnol 19 (6):544-549. doi:10.1016/j.copbio.2008.10.010

Ceja-Navarro JA, Rivera-Orduna FN, Patino-Zúniga L, Vila-Sanjurjo A, Crossa J, Govaerts B, Dendooven L (2010) Phylogenetic and multivariate analyses to determine the effects of different tillage and residue management practices on soil bacterial communities. Appl Environ Microbiol 76 (11):3685-3691. doi:10.1128/AEM.02726-09

Cheeke TE, Coleman DC, Wall DH (2012) Microbial ecology in sustainable agroecosystems. CRC Press,

Conrad R (1996) Soil microorganisms as controllers of atmospheric trace gases (H2, CO, CH4, OCS, N2O, and NO). Microbiological reviews 60 (4):609-640

de Boer W, Folman LB, Summerbell RC, Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development⋆. FEMS Microbiol Rev 29 (4):795-811. doi:10.1016/j.femsre.2004.11.005

Ghani A, Dexter M, Perrott K (2003) Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol Biochem 35 (9):1231-1243. doi:10.1016/S0038-0717(03)00186-X

Giller KE, Beare MH, Lavelle P, Izac AMN, Swift MJ (1997) Agricultural intensification, soil biodiversity and agroecosystem function. Applied Soil Ecology 6 (1):3-16. doi:10.1016/S0929-1393(96)00149-7

Gleason FH, Letcher PM, McGee PA (2004) Some Chytridiomycota in soil recover from drying and high temperatures. Mycol Res 108 (05):583-589. doi:10.1017/S0953756204009736

Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, Ciulla D, Tabbaa D, Highlander SK, Sodergren E, Methé B, DeSantis TZ, Consortium THM, Petrosino JF, Knight R, Birren BW (2011) Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res 21 (3):494-504. doi:10.1101/gr.112730.110

Hartmann M, Widmer F (2006) Community structure analyses are more sensitive to differences in soil bacterial communities than anonymous diversity indices. Appl Environ Microbiol 72 (12):7804-7812. doi:10.1128/AEM.01464-06

Helgason B, Gregorich E, Janzen H, Ellert B, Lorenz N, Dick R (2014) Long-term microbial retention of residue C is site-specific and depends on residue placement. Soil Biol Biochem 68:231-240. doi:10.1016/j.soilbio.2013.10.002

Helgason BL, Walley FL, Germida JJ (2009) Fungal and bacterial abundance in long-term no-till and intensive-till soils of the Northern Great Plains. Soil Sci Soc Am J 73 (1):120-127. doi:10.2136/sssaj2007.0392

Janssen PH (2006) Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Appl Environ Microbiol 72 (3):1719-1728. doi:10.1128/AEM.72.3.1719-1728.2006

Kladivko EJ (2001) Tillage systems and soil ecology. Soil and Tillage Research 61 (1):61-76. doi:10.1016/S0167-1987(01)00179-9

Kurtzman C, Robnett C (1997) Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5'end of the large-subunit (26S) ribosomal DNA gene. J Clin Microbiol 35 (5):1216-1223

Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol Biochem 40 (9):2407-2415. doi:10.1016/j.soilbio.2008.05.021

Lienhard P, Terrat S, Prévost-Bouré N, Nowak V, Régnier T, Sayphoummie S, Panyasiri K, Tivet F, Mathieu O, Levêque J, Maron P-A, Ranjard L (2013) Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland. Agronomy for Sustainable Development:1-9. doi:10.1007/s13593-013-0162-9

Navarro-Noya YE, Gómez-Acata S, Montoya-Ciriaco N, Rojas-Valdez A, Suárez-Arriaga MC, Valenzuela-Encinas C, Jiménez-Bueno N, Verhulst N, Govaerts B, Dendooven L (2013) Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biol Biochem 65:86-95. doi:10.1016/j.soilbio.2013.05.009

Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, Suggests M (2007) The vegan package. Community ecology package

Ramette A (2007) Multivariate analyses in microbial ecology. FEMS Microbiol Ecol 62 (2):142-160. doi:10.1111/j.1574-6941.2007.00375.x

Rueppel ML, Brightwell BB, Schaefer J, Marvel JT (1977) Metabolism and degradation of glyphosate in soil and water. J Agric Food Chem 25 (3):517-528. doi:10.1021/jf60211a018

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl Environ Microbiol 75 (23):7537-7541. doi:10.1128/aem.01541-09

Siciliano SD, Palmer AS, Winsley T, Lamb E, Bissett A, Brown MV, van Dorst J, Ji M, Ferrari BC, Grogan P, Chu H, Snape I (2014) Soil fertility is associated with fungal and bacterial richness, whereas pH is associated with community composition in polar soil microbial communities. Soil Biol Biochem 78 (0):10-20. doi:10.1016/j.soilbio.2014.07.005

Siddiqui ZA, Futai K (2008) Mycorrhizae: sustainable agriculture and forestry. Springer,

Sørensen SR, Ronen Z, Aamand J (2001) Isolation from Agricultural Soil and Characterization of a Sphingomonas sp. Able To Mineralize the Phenylurea Herbicide Isoproturon. Appl Environ Microbiol 67 (12):5403-5409. doi:10.1128/aem.67.12.5403-5409.2001

Team RC (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/,

Yin C, Jones KL, Peterson DE, Garrett KA, Hulbert SH, Paulitz TC (2010) Members of soil bacterial communities sensitive to tillage and crop rotation. Soil Biol Biochem 42 (12):2111-2118. doi:10.1016/j.soilbio.2010.08.006

Zheng Y, Zhang L-M, He J-Z (2013) Immediate effects of nitrogen, phosphorus, and potassium amendments on the methanotrophic activity and abundance in a Chinese paddy soil under short-term incubation experiment. J Soils Sed 13 (1):189-196. doi:10.1007/s11368-012-0601-2

Chapter IV. No favourable effect of reduced tillage

No favourable effect of reduced tillage on microbial community diversity in a silty loam soil (Belgium)

Published in *Agriculture, Ecosystems and Environment*, 2016, 224: 12-21.

Florine Degrune*, Nicolas Theodorakopoulos*, Marc Dufrêne, Gilles Colinet, Bernard Bodson, Marie-Pierre Hiel, Bernard Taminiau, Georges Daube, Carine Nezer, Micheline Vandenbol.

** These authors have contributed equally to this work*

Outline

The aim of this publication was to explore the response of soil microbial diversity to different tillage regimes (conventional and reduced tillage) and crop residue management practices (residue retention and removal) in relation to depth: 0 to 5 cm (top soil) and 15 to 20 cm (deep soil). To infer the structure of microbial communities, we used the method developed in Chapter III.

1. Abstract

Among the soil management practices used to promote sustainable agriculture, reduced tillage and retention of residues from the previous crop are reported to enhance significantly both soil fertility and crop productivity. Here, high-throughput sequencing (454 technology) was used to see how the tillage regime (conventional vs. reduced tillage) and the fate of crop residues (retention or removal) affect microbial communities at two sampling depths (top soil: 0 to 5 cm and deeper soil: 15 to 20 cm) in a fertile silty loam soil in Belgium. All combinations of these three factors were studied. After 6 years of conversion from conventional to reduced tillage, depth emerged as the main factor responsible for variation in microbial diversity, tillage regime ranked second, and finally, crop residue fate had no influence on microbial diversity. For both bacteria and fungi, the diversity appeared higher in the top soil than in the deeper soil, and surprisingly, higher under conventional than under reduced tillage. These differences are explained by changes in community composition due to taxon loss rather than taxon replacement. The specific local set of environmental conditions (a loess-derived soil and an oceanic temperate climate) may explain these results. These observations raise the question: does impoverishment in indicator taxa influence soil processes, and thus crop production? To answer this question, we discuss how the presence of certain indicator taxa liable to play an ecological role might relate to crop productivity.

Keywords

Tillage, crop residue management, 454-pyrosequencing, microbial diversity, indicator taxa

2. Introduction

Agricultural practices, such as the degree of soil disturbance by tillage and the manner in which crop residues are managed, are recognized to influence soil parameters such as water content, temperature, aeration, and the degree of contact between organic matter and mineral soil particles (Kladivko, 2001). Variations in such parameters notably have an impact on soil microbial communities. Soil microbes play essential roles in soil processes (Nannipieri and Badalucco, 2003), providing ecosystem services such as carbon transformation, participation in soil organic matter dynamics, nutrient capture and cycling, and soil structure maintenance (Kibblewhite et al., 2008).

Particular soil management practices, such as reduced tillage, soil protection by means of crop residue retention or soil mulching, crop rotation, and intercropping can significantly enhance both soil fertility and crop productivity in agroecosystems (Scopel et al., 2012). Conservation agriculture based on such practices is recognized as an economically sound, sustainable, and environmentally friendly alternative to conventional agriculture (Hobbs et al., 2008).

Alternative soil management practices are expected to increase soil biodiversity (Clapperton, 2003), thereby improving soil resistance and resilience so as to ensure agroecosystem stability and productivity. Yet agronomists are still far from understanding the impacts of specific practices on microbial communities, their ecological functions, and their ultimate effects on agroecosystems.

Massive parallel DNA sequencing technology is becoming an important tool in microbial ecology (Poisot et al., 2013) for understanding patterns and processes linked to species richness. The high resolution of metagenomic approaches offers insights into the structures of complex microbial assemblages at the level of individual microbial taxa (Cardenas and Tiedje, 2008).

To date, only a few studies have applied metagenomics to investigate the influence of different tillage regimes and types of crop residue management on soil microbial communities (Dorr de Quadros et al., 2012; Lienhard et al., 2013; Navarro-Noya et al., 2013; Sengupta and Dick, 2015). These studies were conducted under specific climates on soils characterized by particular land-use histories. Sengupta and Dick (2015), for example, focused on the native North American prairies, which have a particularly high biological richness, an excellent soil structure, and a very high organic content and microbial biomass. Knops and Tilman (2000) observed that the recent conversion of native grassland to cultivated soil has had detrimental effects on the soil ecosystem, causing loss of some 50 to 80% of the original soil organic matter. As organic matter dynamics is closely related to

microbial activity, the conversion of native grassland to cultivated soil has profoundly changed the diversity and composition of microbial communities (Fierer et al., 2013).

A very different biogeographical and ecological context is to be found in certain areas of Western Europe, such as central Belgium, whose loess-derived soils are among the most fertile in the world and have long been used for intensive agriculture. As pointed out by Rhoton (2000), the context is important, as the influence of a particular tillage regime on soil physical, chemical, and biological properties depends on site characteristics such as soil type, climate, and the number of years since implementation of the tillage system.

To date, no metagenomic study conducted in a sustainable agriculture perspective has yet investigated the response of microbial communities to particular tillage regimes in a loess-derived soil under a temperate oceanic climate. The aim here was to perform such a study on a soil in central Belgium. Specific objectives were to determine diversity levels (based on OTU levels) and changes in microbial community composition (based on taxonomic level, i.e. OTUs are aggregated into taxa) under different combinations of tillage regime (conventional vs. reduced) and crop residue fate (residue removal vs. residues left on the field). As reduced tillage results in two contrasting zones (the first centimeters of soil are mixed each year, while the soil below remains unperturbed), we chose to perform the analysis at two depths: 0 to 5 cm and 15 to 20 cm.

3. Materials and Methods

Site description

The experimental field is located on the experimental farm of Gembloux Agro-Bio Tech (University of Liège, Gembloux, Belgium) characterized by an oceanic temperate climate. According to the World Reference Base (WRB), the soil type is classified as Cutanic Luvisol and is considered one of the most fertile soils in the world: the soil texture is silt loam (FAO) inherited from the loess deposit, with 18-22% clay, 70-80% silt, and 5-10% sand particles, and the organic matter is characterized by a C:N ratio between 10 and 12.

Experimental design and soil treatments

The experimental design and different soil treatments have been applied since autumn, 2008. Before 2008, the site was under conventional tillage. The design of the experimental field consisted of a Latin square arrangement with 16 plots: four soil treatments replicated four times. Each plot is 40 meters long and 15 meters wide. The different soil treatments were as follows: conventional tillage with residue removal (CT/R-, the agricultural practice most commonly used in Belgium for cereals), conventional tillage with residue retention (CT/R+), reduced tillage with residue retention (RT/R+), and reduced tillage with residue removal (RT/R-). The residues removed consisted of harvestable straw, while stubbles and chaffs were left on field in both R+ and R-. In all plots, stubble breaking at a depth of 10 cm was performed to bury the residues. After stubble breaking, plowing to a depth of 25 cm was applied only to the CT plots, with a moldboard plow. Seedbed preparation was identical on all plots and performed at a depth of 7 cm.

Fertilizer, fungicide, and weedkiller treatments were applied equally to each plot (see supplementary data **S2**). Crops are rotated on the studied field and crop history is as follows: *Brassica napus* (2009), *Triticum aestivum* (2010, 2011 and 2012), *Vicia faba* (2013), and *Triticum aestivum* (2014).

Soil sampling

Soil samples were collected from each of the 16 plots in July 2014, at the grain-filling stage. From each plot, six cores were obtained, and from each core two sub-cores were removed: 0 to 5 cm and 15 to 20 cm. The six sub-cores corresponding to the same depth range were pooled and mixed, constituting a composite sample to be used for DNA isolation and soil parameter determinations.

Physico-chemical analysis

In each composite sample, soil physical and chemical properties were determined. Water content was measured by weighing the sample before and after drying it at 105°C. Soil pH was measured in 1 M KCL (2:5 w:v) after two hours of equilibration. Water-extractable elements were quantified by flame absorption (Ca, Mg), flame emission (P, Na), or colorimetry (P) after extraction of 20 g of 8-mm-sieved fresh soil in 100 ml H2O for 1 h at room temperature and filtration on 602 H 1/2. Total organic carbon was quantified by the Walkley-Black method (Nelson and Sommers, 1982) and total nitrogen was quantified by the Kjehldahl method as described by (Bremner and Mulvaney, 1982).

DNA extraction and 454 pyrosequencing of bacterial and fungal genes

DNA was isolated from the soil samples (8 g wet weight) with the PowerMax® soil DNA isolation kit (MO BIO Laboratories, Solana Beach, CA) according to the manufacturer's recommendations. The V1-V3 region of the 16S rRNA (~512 pb) and the D1-D2 region of the 28S rRNA (~680pb) genes were amplified with the help of the following primers: E9-29: 5'-GAGAGTTTGATCATGGCTCAG-3' and E530-541: 5'-ACCGCGGCTGCTGGCAC-3' (Baker et al., 2003) and NL-1; 5'-GCATATCAATAAGCGGAGGAAAAG–3' and NL-4; 5'-GGTCCGTGTTTCAAGACGG-3' (Kurtzman and Robnett, 1997). The reaction mixture contained 5 U FastStart High Fidelity DNA polymerase (Roche Diagnostics, Vilvoorde, Belgium), 1x enzyme reaction buffer, 200 µM dNTPs (Eurogentec, Liège, Belgium), each primer at 0.2 µM, and 100 ng genomic DNA in a final volume of 100 µl. The thermocycling conditions were: denaturation at 94 °C for 15 min followed by 25 cycles of 94 °C for 40 s, 56 °C for 40 s, 72 °C for 1 min, and a final 7 min elongation step at 72 °C. Finally, the 454 pyrosequencing technology (Roche) was used to sequence the PCR products. To maximize

the number of bacterial or fungal sequences per run, the top-soil and deeper-soil samples were run separately.

Processing of 454 pyrosequencing data

The 16S and 28S raw reads were processed with the mothur v.1.35.0 software (Schloss et al., 2009). Reads were trimmed with the following criteria: minimum length: 425 pb; minimum quality score: 25; degree of mismatching allowed: 1 mismatch to the primer and no mismatch to the barcode; homopolymers no longer than 10. Reads with ambiguous bases and singletons were removed. Chimera were checked with Uchime implemented in mothur (Edgar et al., 2011) and removed from the dataset. 16S rDNA sequences were aligned and classified against the SILVA bacterial SSU reference database v119 (Pruesse et al., 2007). For the 28S rDNA sequence alignments, a homemade reference database was built, including 70 LSU reference sequences aligned with the Clustal W alignment tool (Thompson et al., 1994). The sequences were classified against the RDP 28S rRNA database (version 7) (Cole et al., 2009). Denoised sequences were clustered into operational taxonomic units (OTUs) by means of the average neighbor-clustering algorithm implemented in mothur at 97% sequence identity. The numbers of raw reads per run were 75834 (16S rRNA, top soil), 96630 (16S rRNA, deeper soil), 97498 (28S rRNA, top soil), and 73660 (28S rRNA, deeper soil). Rarefaction curves (used to compare the observed richness in different samples, and thus to evaluate the quality of sequencing) were drawn (see supplementary data: **S3** for bacteria and **S4** for fungi) and diversity indexes (richness and Shannon) determined on the basis of the OTUs. The richness and diversity indexes were calculated by normalizing to the number of sequences obtained from the smallest sample. Pyrosequencing raw reads have been deposited in the NCBI Short-Read Archive under accession number SRP061559.

Statistical analyses

All statistical analyses were performed with R software (R Development Core Team, 2011). We used a linear mixed-effect model to assess the influence of the tillage regime, crop residue fate and depth on the α-diversity, i.e. the richness and Shannon diversity indexes (OTU-based). The function lmer of the lmer4 package was used to model α-diversity as a function tillage regime, crop residue fate, and depth as fixed factors and a randomized block design effect as random factor. A likelihood ratio test was used to assess the statistical significance of the fixed factors. The test compares a full model (with the factor of interest, e.g. tillage regime) and a null model (without the

factor of interest). Then, the anova function was used to compare the models on the basis of the p-value.

In addition, a sample-based rarefaction curve analysis was performed with the software *EstimateS* (Colwell, 2005) to compare the cumulative curves of richness (γ-diversity) among groups of 16 samples corresponding to a same depth or tillage regime.

To analyse community composition (β-diversity) we chose the method described by Degrune et al. (2015). Briefly, all of the sequences to which mothur has assigned a genus are recorded with their respective genus affiliations. Among the remaining sequences (those which are unclassified or unknown at genus level), those to which mothur has assigned a family (the rank just above genus) are recorded with their family affiliation, and so on up to the highest rank (phylum). The only OTUs assigned to a taxonomic rank above the genus are ones that are unclassified or unknown at a lower rank. For example, Acidobacteria contains only those OTUs whose taxonomic affiliation below the phylum rank is unknown or unclassified.

Differences in community composition (β-diversity) were assessed using distance-based redundancy analysis of the Bray-Curtis distance matrix, using the function capscale of the vegan package (Oksanen et al., 2007). The anova function with a permutation test was used to test the significance (9999 permutations). We assumed that differences in community composition are mainly due to the presence of representative taxa found under each set of soil conditions determined by the tillage regime, the crop residue fate, and the depth. The function Indval of the labdsv package was used to identify these indicator taxa. Indicator taxa were identified on the basis of an indicator value which is a combination of the abundance of a taxon in the target group compared to other groups (specificity) and its relative frequency of occurrence in that particular group (fidelity) (Dufrêne and Legendre, 1997).

Finally, we related the microbial pattern to environmental parameters. The function envfit was used to fit the physico-chemical parameters to the ordination graph: the displayed arrows show the directions of the soil physico-chemical gradients, while the length of each arrow is proportional to the correlation between the variable and the ordination (Oksanen et al., 2007). The function vectorfit was used to test the significance of the correlations observed.

4. Results

Phylum composition

Whatever the depth (0 to 5 cm or 15 to 20 cm), tillage regime (CT or RT), or fate of crop residues (R+ or R-), the bacterial community of the soil proved to be dominated by Proteobacteria (25%-31%), Acidobacteria (15%-23%), and Bacteroidetes (15%-25%) and the fungal community by Ascomycota (48%-67%) and Basidiomycota (15%-25%) (**Figure 19**).

Impacts on microbial alpha and gamma diversity

On the basis of the likelihood ratio test, the results (**Table 5**) showed that tillage regime and depth affected the microbial α-diversity, while no effect of crop residue fate was observed. We also tested for interactions between depth and the random factors, and between depth and the fixed factors, and observed no interaction effects (results not shown). **Table 6** shows the bacterial and fungal α-diversity levels for each factor of variation. **Figure 20** shows the levels of bacterial (a) and fungal (b) α-diversity for conventional (CT) and reduced tillage (RT) and for the top soil (A) and deeper soil (B).

The comparison of γ-diversity of top-soil samples with that of the deeper-soil, and the γ-diversity of the RT samples with that of CT samples are shown in the **Figure 21**. The first point of each of these curves shows the mean richness per sample (α-diversity) in the studied group of 16 samples, while the last point shows the total richness (γ-diversity) of the whole group, and points 2, 3..15 show the mean richness (γ-diversity) of many random combinations of 2, 3...15 samples. Such a curve tends to be rather flat if the samples in the investigated set contain many common, readily detectable OTUs, but it rises steeply if the different samples contain more specific and/or rare OTUs. Hence, effects observed at the level of α-diversity can disappear or even be reversed at the level of γ-diversity. Our results confirm at cumulative γ-diversity level the effects of depth and tillage regime observed at α-diversity level: in all four presented plots, the distance between curves either remains approximately constant or increases from left to right. In the latter case, more new sample-specific OTUs continue to be detected in one group than in the other as the number of samples considered increases.

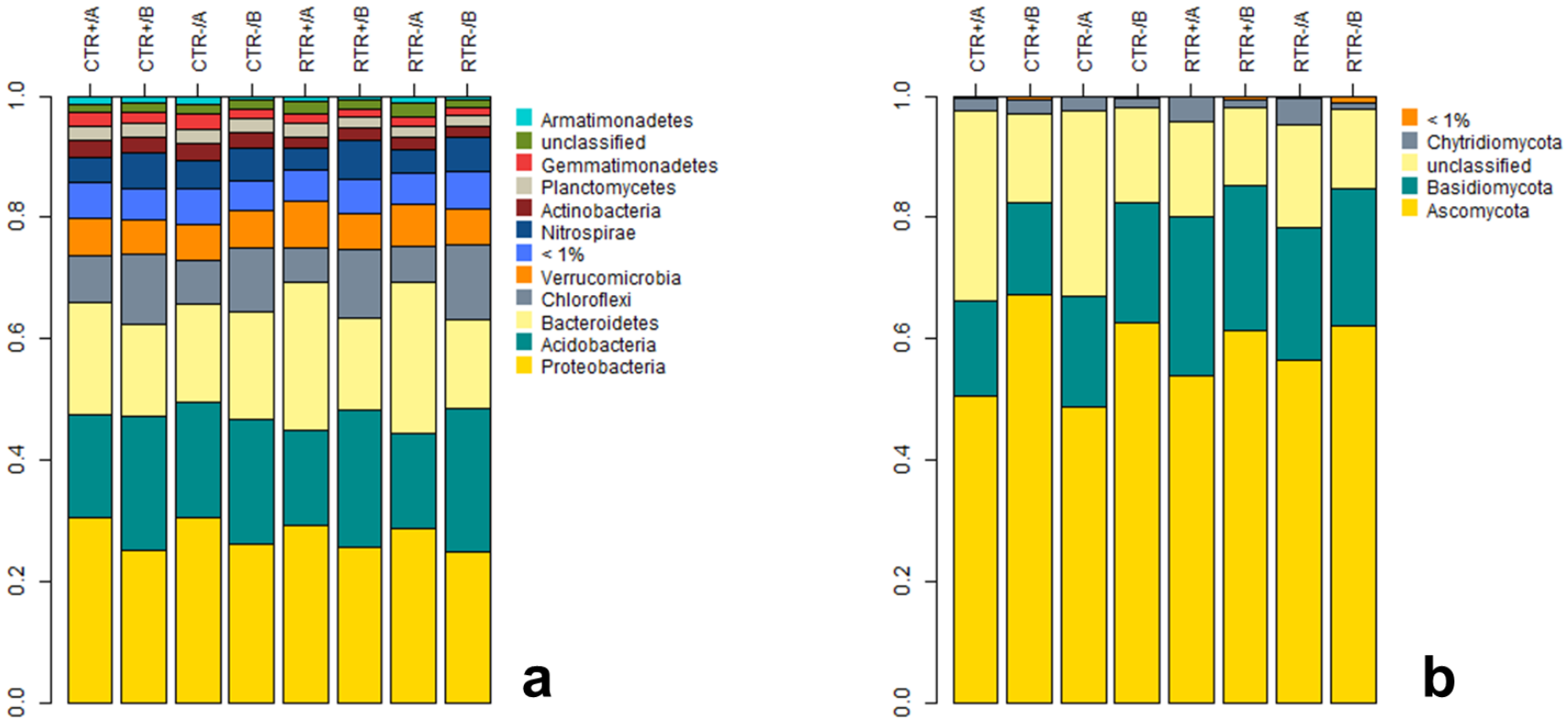


Figure 19 Bar plot representation of the relative abundances of (a) bacterial and (b) fungal phyla in the soil according to the depth and treatment applied. Soil treatments are defined by RT for reduced tillage, CT for conventional tillage, R+ for crop residue retention and R- for residue removal. Depths are defined by A for 0–5 cm and B for 15–20 cm.

Table 5 Results of the Likelihood Ratio Test, i.e. the comparison of two linear mixed models, the null model without the factor of interest and the full model with the factor of interest in order to detect the effect of the factors on the a-diversity indexes.

		Factors of variation		
		Tillage regime	Crop residue fate	Depth
Bacteria	Richness	$\chi^2(1)=8.97$, $p<0.01$	$\chi^2(1)=2.24$, NS	$\chi^2(1)=20.59$, $p<0.001$
	Shannon	$\chi^2(1)=4.38$, $p<0.05$	$\chi^2(1)=0.13$, NS	$\chi^2(1)=29.27$, $p<0.001$
Fungi	Richness	$\chi^2(1)=25.88$, $p<0.001$	$\chi^2(1)=2.19$, NS	$\chi^2(1)=46.42$, $p<0.001$
	Shannon	$\chi^2(1)=24.10$, $p<0.001$	$\chi^2(1)=2.19$, NS	$\chi^2(1)=36.54$, $p<0.001$

Table 6 Means and standard deviations of the α-diversity indexes (richness and Shannon) of bacteria and fungi according to the factors of variation.

Alpha diversity indexes		Factors of variation					
		Tillage regime		Crop residue fate		Depth	
		CT	RT	R+	R-	A	B
Richness	Bacteria	873 ± 64^a	820 ± 70^b	851 ± 57^a	843 ± 84^a	891 ± 35^a	802 ± 71^b
	Fungi	440 ± 64^a	$382\pm54_b$	418 ± 72^a	405 ± 60^a	460 ± 47^a	363 ± 40^b
Shannon	Bacteria	6.2 ± 0.2^a	6.1 ± 0.2^b	6.19 ± 0.18^a	6.17 ± 0.25^a	6.34 ± 0.06^a	6.02 ± 0.2^b
	Fungi	4.9 ± 0.2^a	4.7 ± 0.2^b	4.80 ± 0.28^a	4.75 ± 0.23^a	4.96 ± 0.20^a	4.6 ± 0.18^b

For each line and each factor of variation, different letters mean the values are significantly different. Soil treatments are defined by RT for reduced tillage, CT for conventional tillage, R+ for crop residue retention and R- for residue removal.

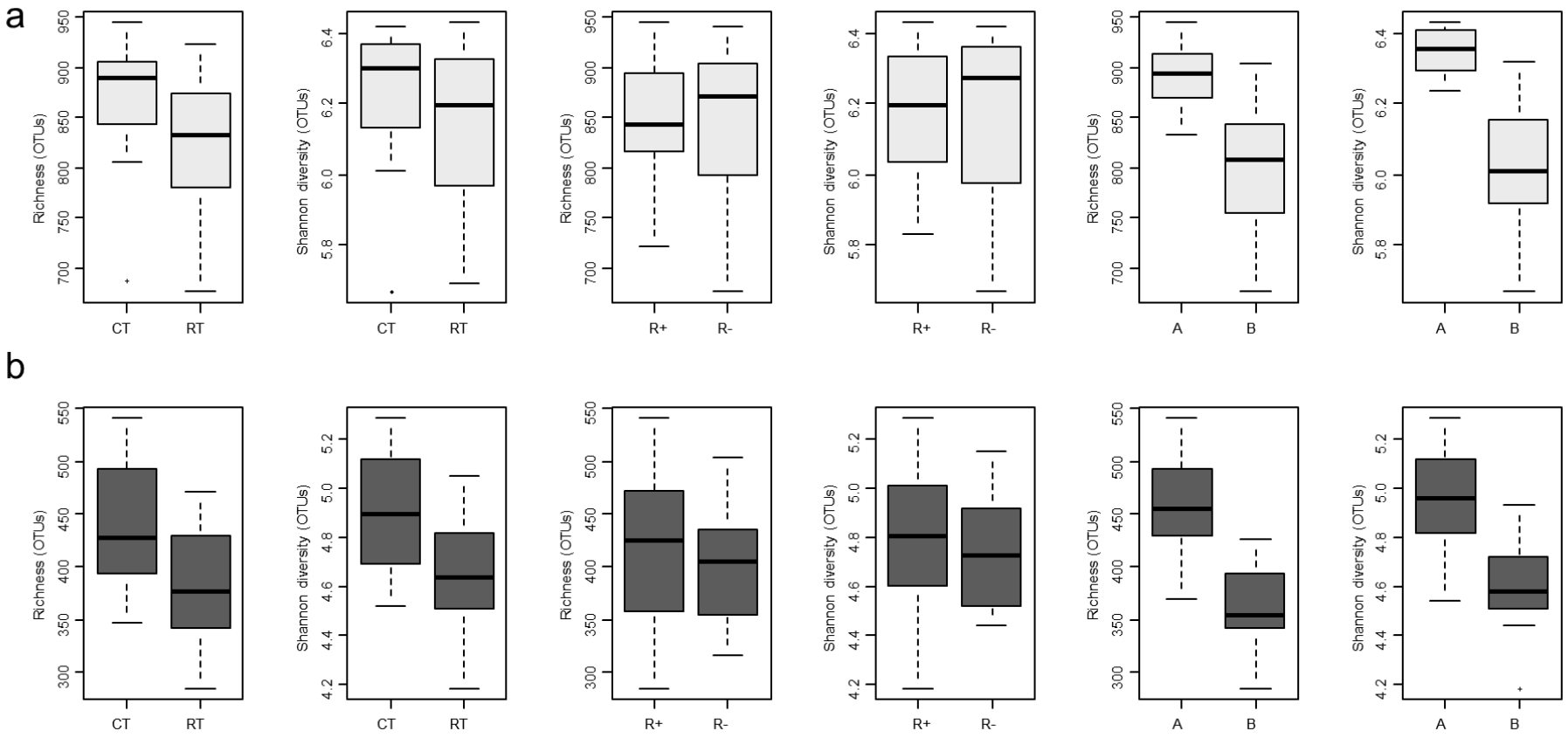


Figure 20 Boxplots of operational taxonomic units (OTUs) richness and Shannon diversity index for bacteria (a) and fungi (b). Soil treatments are defined by RT for reduced tillage, CT for conventional tillage, R+ for crop residue retention and R- for residue removal. Depths are defined by A for 0–5 cm and B for 15–20 cm.

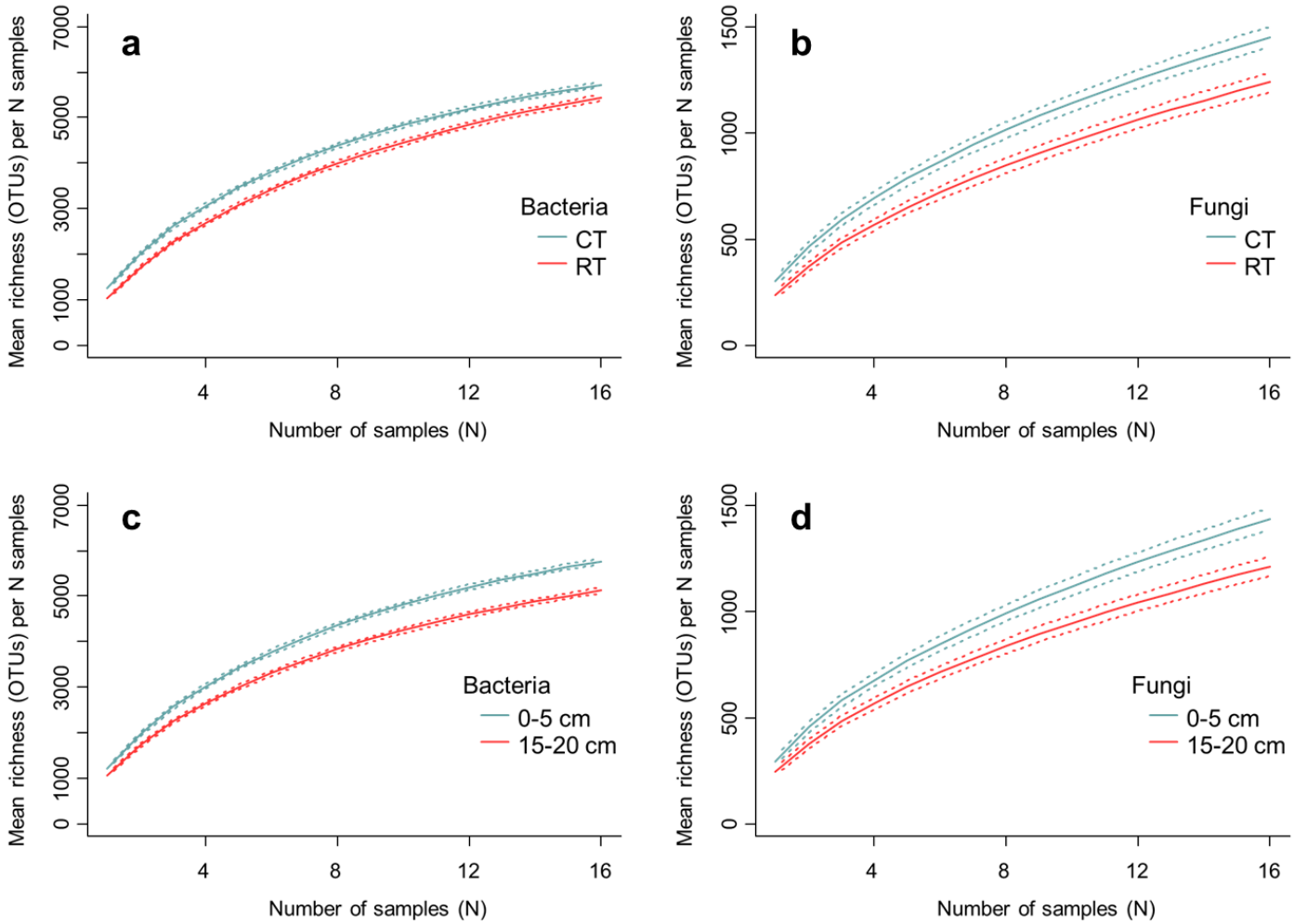


Figure 21 Sample-based rarefaction curves drawn to compare the γ-diversity between conventional (CT) and reduced tillage (RT) for (a) bacteria and (b) fungi, and to compare the γ-diversity between the top soil (0–5 cm) and deeper soil (15–20 cm) for (c) bacteria and (d) fungi.

Impacts on microbial community composition

The influence of tillage regime, depth, and crop residue fate on microbial community composition (β-diversity) was assessed by constrained ordination analysis (db-RDA, Bray-Curtis distance). In other words, the only variations in the taxon table that were displayed and analyzed were those which could be explained by tillage regime, depth, or crop residue fate. On the basis of the method described by Degrune et al. (2015), 356 bacterial and 176 fungal taxa were identified and used to build the taxon table.

Depth emerged as the main factor responsible for microbial community variance, explaining respectively 20% and 11% of the bacterial and fungal community variance. Tillage regime ranked second, explaining respectively 7% and 8% of the bacterial and fungal community variance. Finally, crop residue fate had no influence on community composition (**Table 7**).

The results of the ordination analysis are displayed in **Figure 22a** & **Figure 23a**. The ordination graphs based on the relative abundances of bacterial (**Figure 22a**) or fungal (**Figure 23a**) taxa were constrained with the only two significant effect factors: tillage regime and depth. Both graphs display the similarity (or dissimilarity) of community composition between samples. Samples having a highly similar community composition appear close to each other, and the distance between samples increases with the difference in community composition. Figures show that our ordination analyses distinguish, on the basis of bacterial or fungal community composition, four groups of samples: “CT, 0-5 cm”, “CT, 15-20 cm”, “RT, 0-5 cm”, and “RT, 15-20 cm. For bacteria (**Figure 22a**), the greatest difference in community composition occurs in the top soil, between CT and RT (green triangles). For fungi (**Figure 23a**), the greatest difference occurs under RT, between the top soil and the deeper soil (full triangles).

Table 7 Results of the constrained ordination analysis (db-RDA using the Bray-Curtis distance) for bacteria and fungi.

	Bacteria		Fungi	
	% of variance explained	P_{value}	% of variance explained	P_{value}
Depth (CAP1)	20.2	*** <0.001	11.1	*** <0.001
Tillage regime (CAP2)	7.1	*** <0.001	8.5	*** <0.001
Crop residue fate (CAP3)	2.3	NS	2.8	NS

Depth and tillage regime are the two main factors explaining bacterial and fungal community variation.

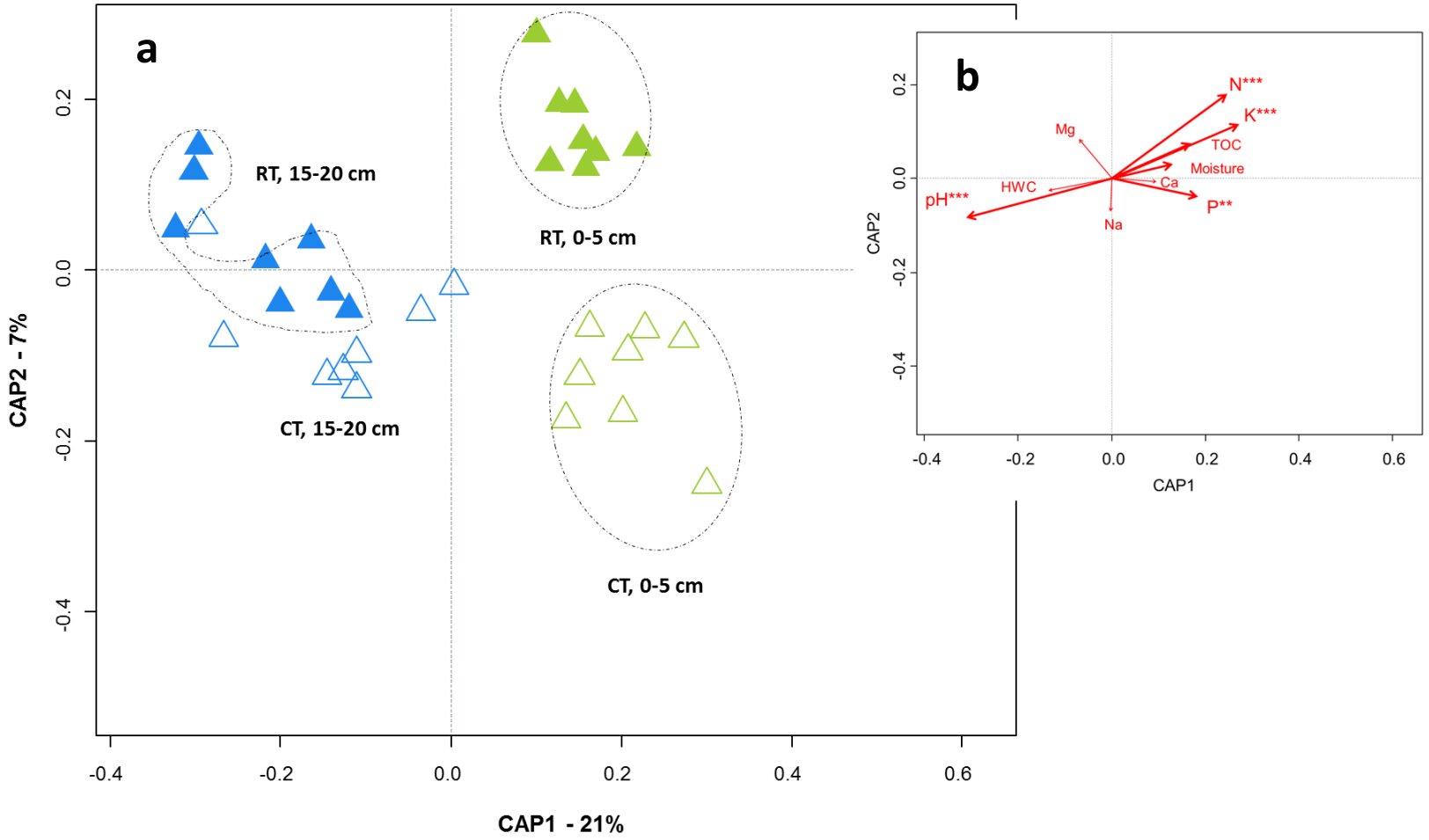


Figure 22 (a) Constrained ordination analysis of Bray-Curtis distances based on the relative abundances of bacterial taxa for each sample. The axes CAP1 and CAP2 explain, respectively, 21% and 7% of the community variation. Each triangle corresponds to a sample, identified by color coding and symbol filling. Four groups of samples can be distinguished: green, empty: CT, 0–5 cm; blue, empty: CT, 15–20 cm; green, full: RT, 0–5 cm; blue, full: RT, 15–20 cm. CT = conventional tillage and RT = reduced tillage. (b) Correlation between physico-chemical variables and samples: N = nitrogen, K = potassium, TOC = total organic content, P = phosphorus, Mg = magnesium, HWC = hot water carbon, Ca = calcium, Na = sodium. Variables with stars are significantly correlated to the samples. Significance levels are as follows: ** significant at $p < 0.01$; *** significant at $p < 0.001$.

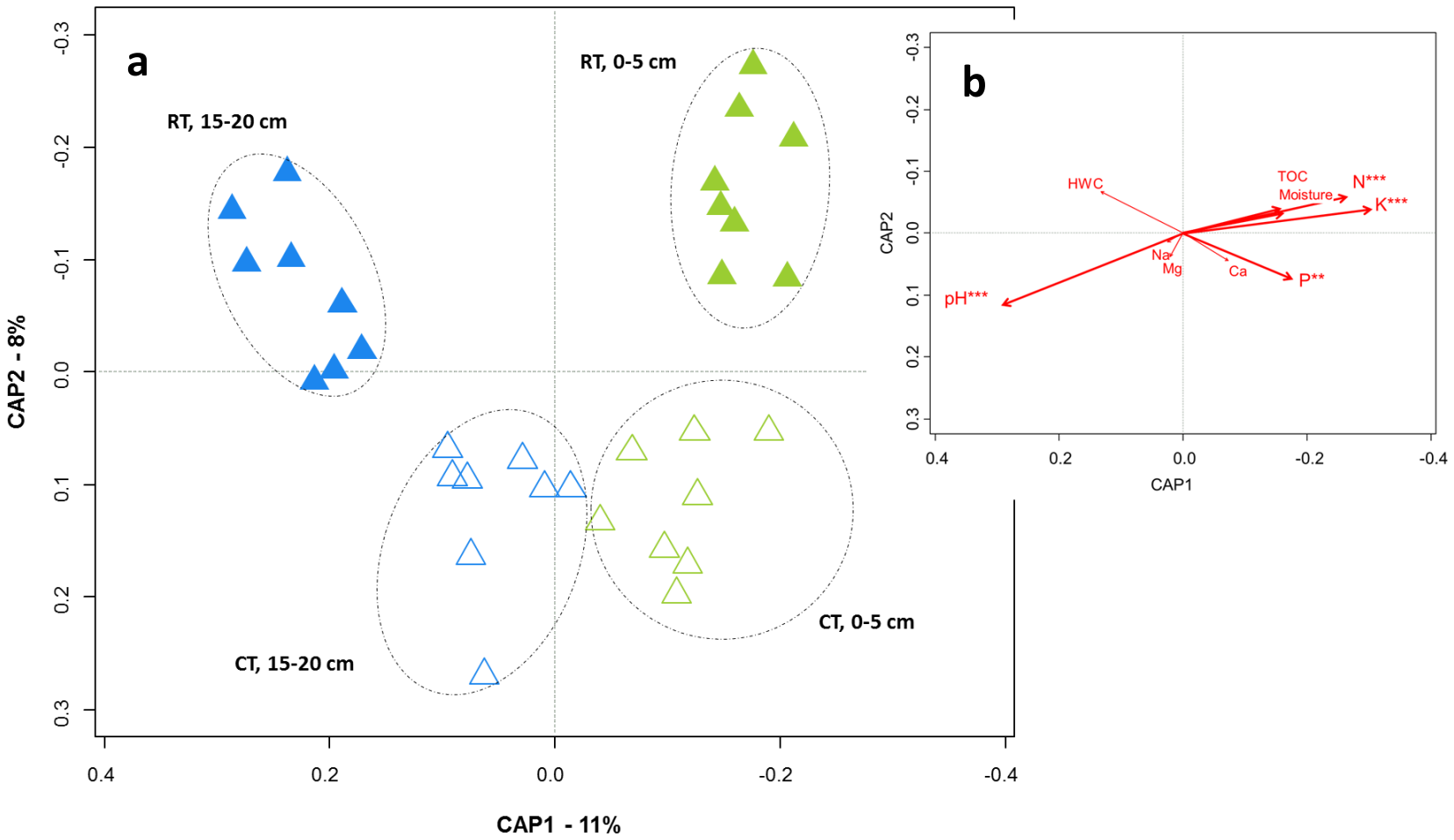


Figure 23 (a) Constrained ordination analysis of Bray-Curtis distances based on the relative abundances of fungal taxa for each sample. The axes CAP1 and CAP2 explain, respectively, 21% and 7% of the community variation. Each triangle corresponds to a sample, identified by color coding and symbol filling. Four groups of samples can be distinguished: green, empty: CT, 0–5 cm; blue, empty: CT, 15–20 cm; green, full: RT, 0–5 cm; blue, full: RT, 15–20 cm. CT = conventional tillage and RT = reduced tillage. (b) Correlation between physico-chemical variables and samples: N = nitrogen, K = potassium, TOC = total organic content, P = phosphorus, Mg = magnesium, HWC = hot water carbon, Ca = calcium, Na = sodium. Variables with stars are significantly correlated to the samples. Significance levels are as follows: ** significant at $p < 0.01$; *** significant at $p < 0.001$.

Link between samples and soil physico-chemical parameters

We then sought to relate the observed patterns of community composition (**Figure 22a** & **Figure 23a**) to environmental variables. **Figure 22b** & **Figure 23b** show the correlations between samples and soil physico-chemical parameters, and provide an overview of physico-chemical gradients in the experiment. We found the pH, nitrogen, potassium, and phosphorus to correlate significantly with community composition. They might thus explain the microbial patterns observed. The values (means and standard deviations) of the soil parameters are summarized in the **Table 8**. As shown in the correlation graphs, the pH correlated positively with the composition of deeper-soil samples, while N, K, and P correlated positively with top-soil sample composition.

Microbial indicator taxa

It thus appears that depth and tillage regime have a strong influence on the microbial community composition. Such differences can reflect turnover, i.e. the replacement of one taxon by another, or nestedness, i.e. the fact that one assemblage is a subset of another (Baselga, 2010). We assumed that the observed pattern is mainly due to the presence of specific taxa which are sensitive to the set of soil conditions created by the tillage regime, the crop residue fate and the depth. Such representative taxa or "indicator taxa" were identified using the Indval function. Of the 356 bacterial and 176 fungal taxa identified, respectively 50% and 34% proved to be indicative of a particular set of experimental conditions (**Figure 24**).

The number of bacterial indicator taxa identified was higher under CT (61) than under RT (6). This shows that six years after conversion from CT to RT, the RT plots showed a clear impoverishment in bacterial taxa ("taxon loss" sensu Baselga (2010)). For fungi, the trend was weaker but observable: the number of indicator taxa found under CT (19) was again higher than the number found under RT (8). We also detected a difference due to depth: the number of bacterial indicator taxa was higher in the top soil, which means that the deeper soil was impoverished in bacterial taxa. For fungi, in contrast, Indval analysis revealed fewer indicator taxa in the top soil than in the deeper soil.

Table 8 Means and standard deviations of the soil physical and chemical parameters according to the factors of variation.

Soil parameters		**Factors of variation**					
		Tillage regime		**Crop residue fate**		**Depth**	
		CT	RT	R+	R-	A	B
pHKCL	-	6.2±0.5	6.2±0.5	6.3±0.6	6.1±0.4	5.8±0.3	6.6±0.4
TOC	%	1.3±0.2	1.4±0.2	1.4±0.26	1.2±0.15	1.4±0.2	1.3±0.2
N		0.13±0.01	0.13±0.02	0.13±0.01	0.13±0.01	0.14±0.01	0.12±0.01
Soil moisture		20±2	20±2	20±2	20±2	21±1	19±3
HWC	mg/Kg	324±105	344±99	353±97	315±104	301±91	367±102
P		3.7±1.1	3.4±1.2	3.7±1.1	3.4±1.2	4.0±1.1	3.1±1.0
Mg		3.1±0.9	3.1±0.8	3.2±0.5	3.0±1.1	3.0±0.6	3.2±1.0
Na		25.8±2.3	25.1±3.9	25±2.7	26±3.6	26±3.2	25±3.3
K		6.6±3.0	8.0±6.5	7.6±5.5	7.0±4.8	10.4±5.3	4.2±2.2
Ca		45±23	40±23	45±25	39±21	48±27	36±15

For each line and each factor of variation, different letters mean the values are significantly different. CT = conventional tillage and RT = reduced tillage.

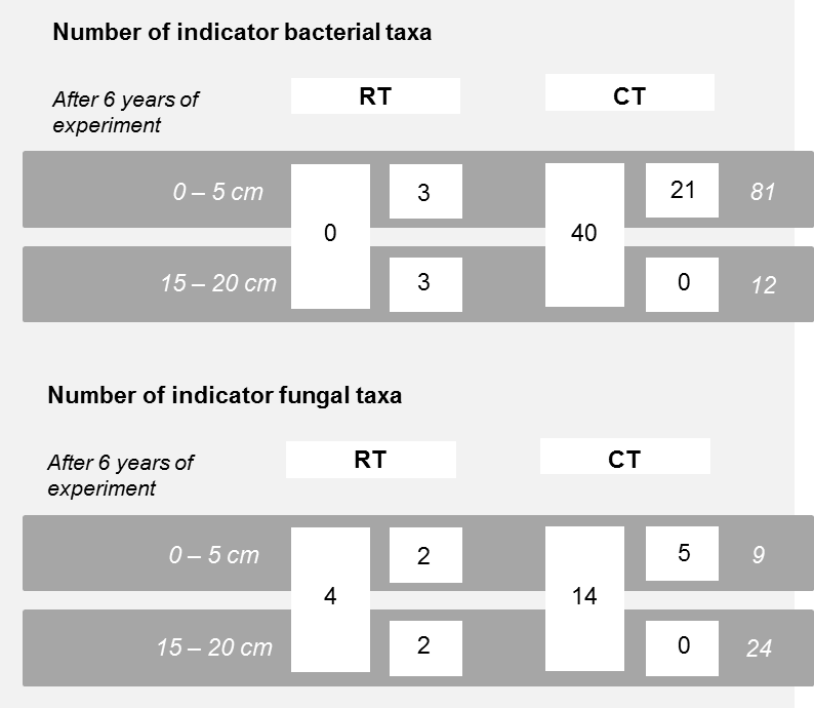


Figure 24 Numbers of bacterial and fungal indicator taxa found in the Indval analysis. CT = conventional tillage, RT = reduced tillage.

5. Discussion

The present study, based on high-throughput sequencing, has focused on the influence of tillage, crop residue fate, and depth on the diversity and composition of bacterial and fungal communities in an agricultural soil under a temperate oceanic climate.

No effect of crop residue retention/removal

We have observed here no influence of crop residue retention/removal on microbial richness or community composition under any tillage regime. Only a few studies have used high-throughput technologies to investigate the influence of both tillage regime and crop residue fate on microbial communities. Ceja-Navarro et al. (2010) report highest levels of bacterial diversity under conditions of zero tillage and crop residue retention. In another study, this team also observed a greater influence of crop residue management on bacterial communities when no tillage was applied than under conventional tillage (Navarro-Noya et al., 2013). They concluded that the soil bacterial community depends strongly on both the tillage regime and the crop residue management practice. These studies, however, focused on regions with environmental conditions very different from those affecting our experimental field, making it hard to compare their results with ours. Worth mentioning, for example, are the different climates (semi-arid vs. temperate oceanic), different soil management histories (recent conversion of grassland or forest to cultivated land vs. longstanding cultivation), and the type of soil, which in our study is of particular interest (silt loam inherited from loess deposits). Here, the high level of soil fertility may explain the absence of any effect of crop residue retention/removal on microbial communities: soil amendment with crop residues could be negligible as compared to the total amount of organic carbon initially present.

Effects of depth and tillage regime

We have recorded higher bacterial and fungal richness values in the top soil (0 to 5 cm) than in the deeper soil (15 to 20 cm). Rahman (2008) and Eilers (2012) have likewise found bacterial and fungal diversity to be highest in the top 10 cm. Other authors have also noted a decline, with increasing depth, in the activity and abundance of functional groups such as nitrate-reducing bacteria (Marhan et al., 2011) and mycorrhizal fungi (Bahram et al., 2015).

The present data further reveal an influence of the tillage regime on soil microbial communities. Surprisingly, for both bacteria and fungi, we observe significantly lower richness and Shannon diversity indexes in the less disturbed soil (reduced tillage) than in the more perturbed soil (conventional tillage). For bacteria, we hypothesized that soil aggregate disruption caused by plowing (Paustian et al., 2000) releases organic matter, making it available for bacterial activity. One hypothesis for fungi could be the physical disturbance caused by plowing, which creates new spaces for colonization by minor or new fungal species (Denslow et al., 1985; Tilman, 1982).

To date, only a few teams have used high-throughput sequencing technology to investigate the influence of the tillage regime on the microbial diversity (Dorr de Quadros et al., 2012; Lienhard et al., 2013; Navarro-Noya et al., 2013; Sengupta and Dick, 2015). Overall, these authors observed higher microbial diversity under less perturbing regimes such as reduced or no tillage. These experiments, however, focused on soils characterized by very specific environmental conditions (Brazil, Laos, USA), making it hard to compare their results with ours, as mentioned above. Therefore, given the great potential (high fertility) of the soil in the region on which we have focused, more studies are needed to understand relationships between microbial communities and agricultural practices in this particular region.

Link between microbial diversity and crop productivity

Most soil processes, such as nutrient cycling, are mediated by microorganisms (Nannipieri and Badalucco, 2003). Microbes influence soil fertility and are thus liable to influence crop productivity. This explains current interest in characterizing microbial biodiversity.

Here, although lower richness and diversity were associated with reduced tillage, no significant difference in wheat yield was recorded between the two tillage regimes. The yields were 9.06±0.30 and 8.94±0.24 t/ha for conventional and reduced tillage, respectively. It thus seems that the observed reduction in species number did not alter the microbe-mediated soil processes essential to crop growth and health. The functional redundancy of soil microorganisms is particularly high (Nannipieri et al., 2003), i.e. different species can have the same function in an ecosystem (Loreau, 2004). According to Schimel (1995), functional redundancy is greater for “broad” processes such as respiration and mineralization, carried out by a large and diverse group of microorganisms, than for “narrow” processes carried out by a more restricted group of microorganisms. The high functional redundancy of soil microorganisms probably explains why the slight decrease in microbial richness

recorded here had no observable repercussions on soil functions and ultimate crop productivity. To date, how and to what extent changes in microbial diversity affect soil ecosystem functioning and stability remains controversial. Effects on soil process rates depend on which species are removed from the community and to what extent the remaining species can compensate for their absence. It would appear that ecosystem functions depend on species traits and changes in community composition rather than on species richness per se (Bardgett, 2002).

Effects on microbial community composition

The present study has evidenced effects of depth and tillage regime on microbial community composition. Our constrained ordination analysis has indeed highlighted four distinct groups of samples, corresponding to four sets of experimental conditions: CT at 0 to 5 cm, CT at 15 to 20 cm, RT at 0 to 5 cm, and RT at 15 to 20 cm.

We have further used Indval analysis to determine to what extent the observed variations in taxon composition reflect the presence of taxa specifically associated with each group ("taxon replacement" sensu Baselga (2010)) or an impoverishment of the microbial community ("taxon loss" sensu Baselga (2010)). This analysis has clearly evidenced an impoverishment in both bacteria and fungi under RT, as the number of indicator taxa is higher under CT (**Figure 24**). We conclude that conversion from conventional tillage to reduced tillage has led, over a six-year period, to an overall impoverishment in bacterial and fungal taxa.

These results raise the question: does impoverishment in indicator taxa influence soil processes, and thus crop production? To answer this question, it is necessary to know the ecological roles played by the indicator taxa. In the next section, we discuss how the presence of certain indicator taxa liable to play an ecological role might relate to crop production.

Putative ecological roles of indicator taxa

Among the bacterial (160) and fungal (60) indicator taxa identified (see supplementary data **S5** for bacteria and **S6** for fungi), the large majority remains poorly described in terms of functional capabilities. For a few of them, however, a putative role in agroecosystem functioning and a potential impact on crop productivity have been proposed. For example, we found *Flavobacterium*, *Mesorhizobium*, *Nirtosospira*, *Pseudomonas*, and *Gibberella* to be indicators of the top soil, while *Nitrospira*, *Glomus*, and *Penicillium* emerged as deeper-soil indicators (**Figure 25**). *Paenibacillus* was found to be an indicator of the deeper soil under RT, while Arthrobacter and Streptomyces were found to be indicators of conventional tillage (**Figure 25**).

On the basis of current knowledge available in the literature, we found members of the genera *Pseudomonas*, *Flavobacterium*, *Streptomyces*, *Paenibacillus*, and *Arthrobacter* to be well-known plant growth promoting rhizobacteria (PGPRs), capable of stimulating plant growth by facilitating uptake of certain nutrients from the environment and through their pest- and pathogen-suppressing action (Glick, 1995; Govindasamy et al., 2011). *Nitrosospira* and *Mesorhizobium* members are also of functional interest in agriculture, being involved in the nitrogen cycle (Francis et al., 2007; Gage, 2004). *Glomus* and *Penicillium* members are of particular interest, because arbuscular mycorrhizal (AM) fungi are known to form a symbiotic relationship with plant roots. Olsson et al. (1997) and Nilsson et al. (2007) report a decrease in AM fungus abundance with increasing availability of nutrients such as nitrogen and phosphorus. Here, the deeper-soil samples displayed a lower nutrient status (phosphorus, potassium, nitrogen, and total organic content) than the top soil. Koorem et al. (2014) state that the low nutrient status of certain soil habitats induces plants to interact with soil organisms such as AM fungi. Lastly, the genus *Gibberella* includes strains known to be beneficial to plants and others known to be deleterious. An example of the latter is *Gibberella zeae* (also called *Fusarium gramineum*), a fungal pathogen responsible for head blight of wheat (Bottalico and Perrone, 2002). The putative roles of the other indicator taxa remain unknown, because most of them cannot be cultured in the laboratory. Although microorganisms play crucial roles in soil processes, to date there is a huge lack of knowledge on their roles in agro-ecosystems.

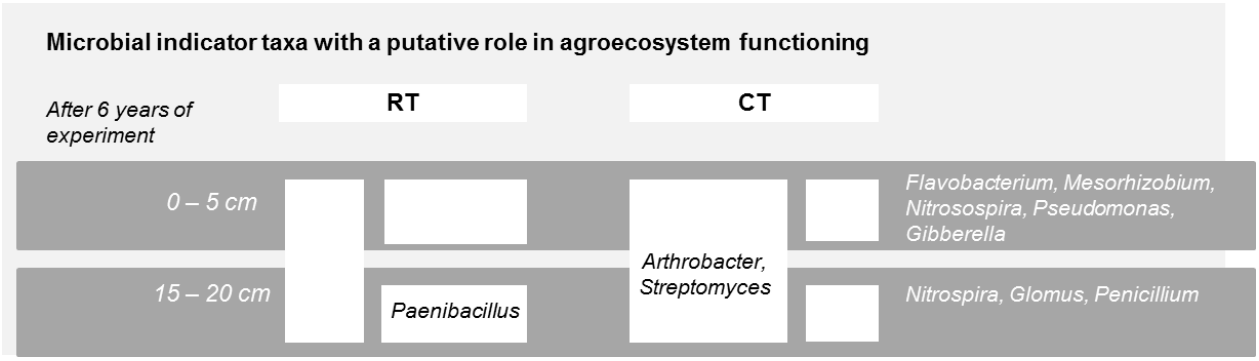


Figure 25 Example of indicator taxa liable to play an ecological role influencing crop productivity. CT = conventional tillage, RT = reduced tillage.

6. Conclusion

The present study, conducted on a highly fertile silt loam soil under a temperate climate, reveals effects of both tillage regime and depth on microbial community composition. In particular, it reveals a notable decrease in microbial diversity (richness per se and evenness) under RT. We further confirm that the change is an impoverishment, since six years after conversion from CT to RT, a number of taxa indicative of CT have disappeared and have not been replaced by RT-specific taxa. We thus conclude that reduced tillage has not favoured microbial diversity over this period. On the other hand, we have observed no effect of crop residue fate (retention or removal) on microbial diversity and no difference in crop productivity between CT and RT. We believe that to confirm our results, other studies should be conducted under similar environmental conditions, i.e. temperate oceanic climate, a soil that is very fertile and has been cultivated for a long time. Lastly, to increase knowledge about the ecological roles played by microbial taxa identified in soil samples, there is a need to associate taxonomic analyses with functional ones in order to better understand the influence of agriculture on soil functioning.

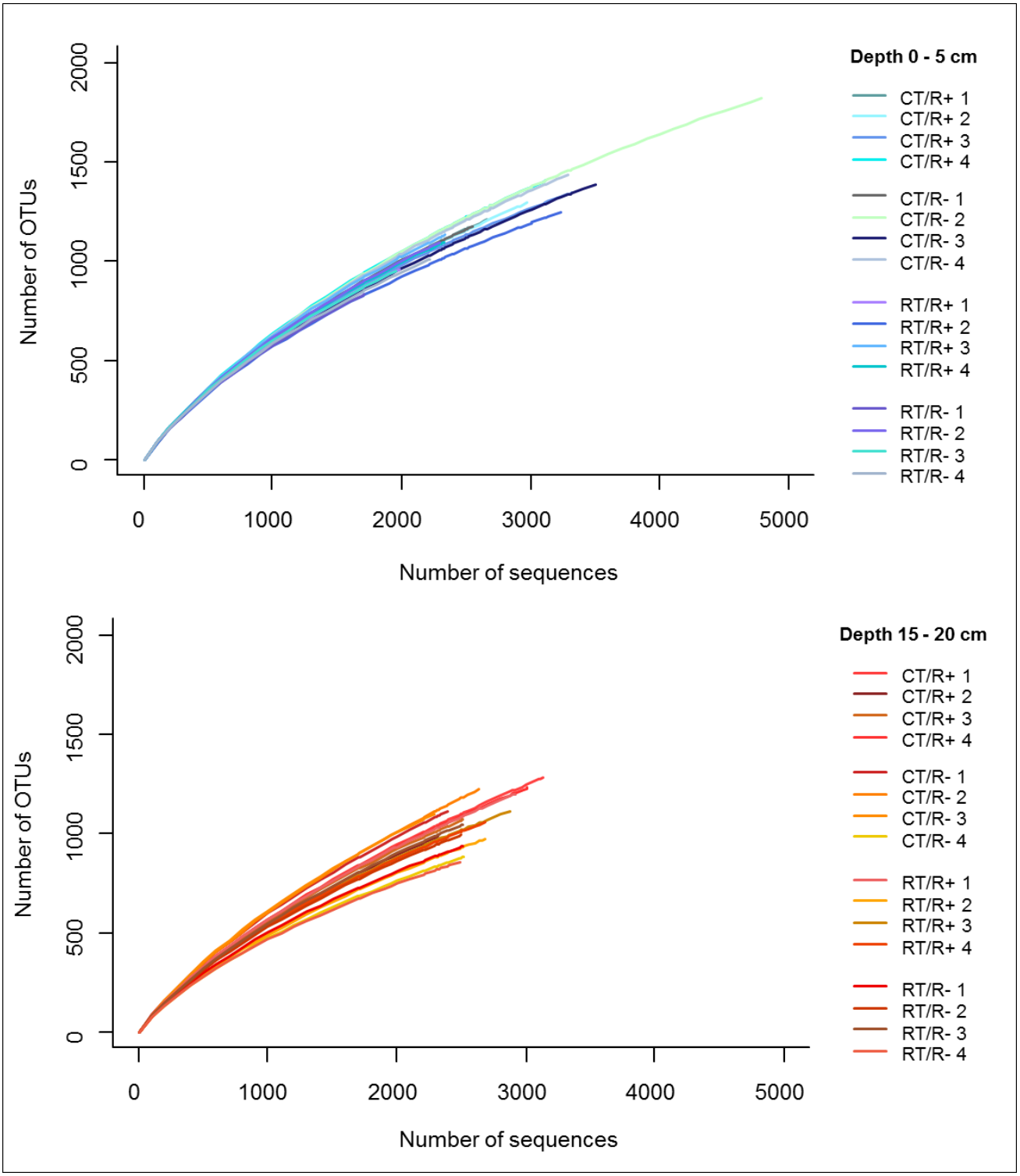
Acknowledgments

We thank the University of Liège-Gembloux Agro-Bio Tech, and particularly the AgricultureIsLife research platform, for funding this research project. We also thank Yves Brostaux for his statistical help and Jean-Charles Bergen for his technical assistance.

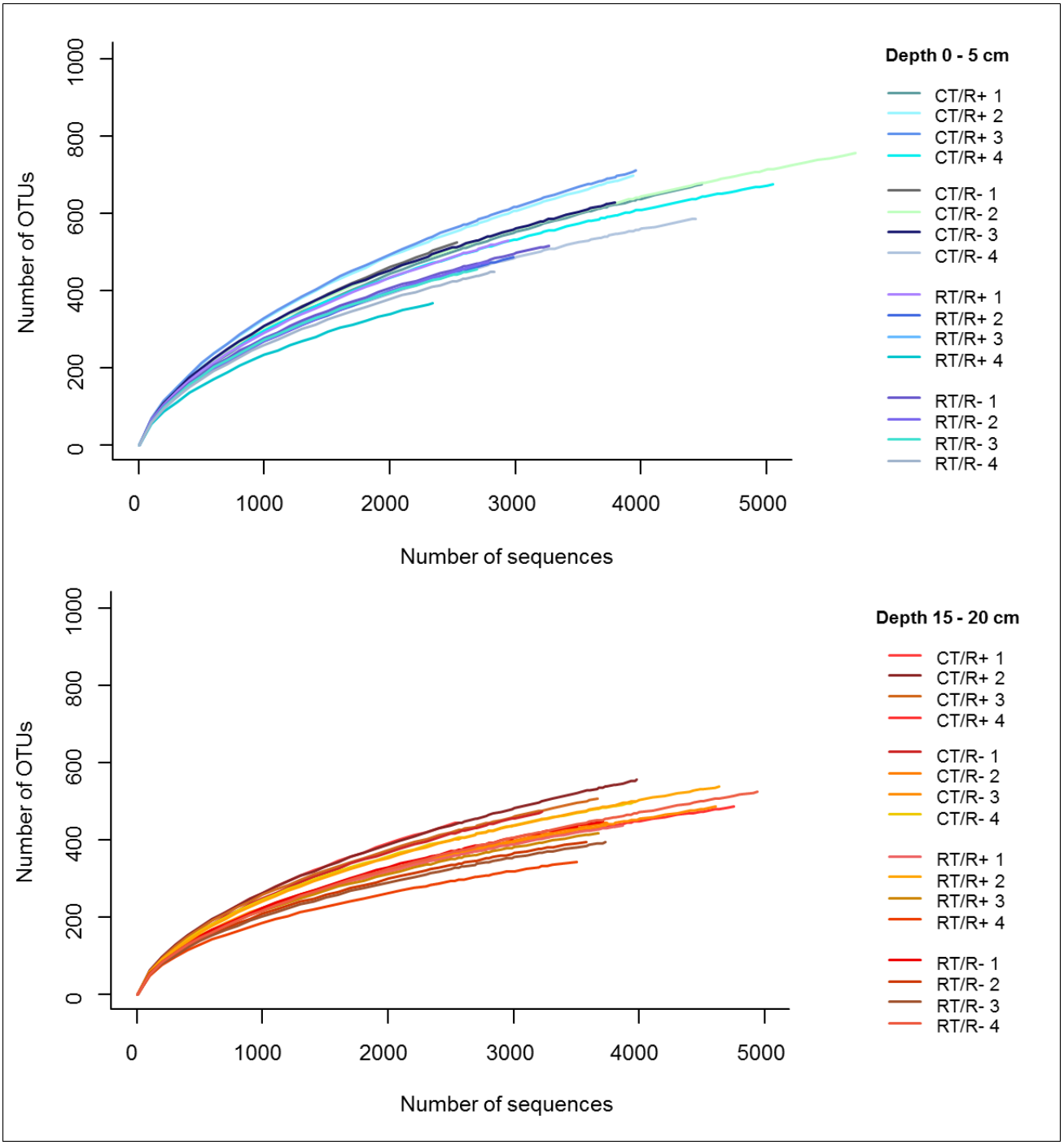
7. Supplementary material

Dates of application	Treatment	Product name	Conc/ha	Active substances
11/03/2014	Nitrogen fertilization	Liquid Nitrogen 39 %	95.07 L	
1/04/2014	Weed killer	ATLANTIS	0.3 kg	MEFENPYR-DIETHYL (9%), IODOSULFURON-METHYL-SODIUM (0.6%), MESOSULFURON-METHYL (3%)
		HUSSAR ULTRA	0.10 L	MEFENPYR-DIETHYL (300 g/l), IODOSULFURON-METHYL-SODIUM (100 g/l)
		ACTIROB B	1 L	Esterified rapeseed oil (812 g/l)
15/04/2014	Nitrogen fertilization	liquid Nitrogen 39 %	157.73 L	
15/04/2014	Growth regulator	CYCOFIX 750	1.02 L	CHLOORMEQUAT (750 g/l)
25/04/2014	Weed killer	AXIAL	1.47 L	CLOQUINTOCET-MEXYL (12.5 g/l) and PINOXADEN (50 g/l)
27/04/2014	Fungicide	OSIRIS	2.03 L	EPOXYCONAZOL (37.5 g/l) and METCONAZOL (27.5 g/l)
12/05/2014	Nitrogen fertilization	liquid Nitrogen 39 %	181.54 L	
16/05/2014	Weed killer	ALLIE	30.55 g	METSULFURON-METHYL (20%)
6/06/2014	Fungicide	AVIATOR	1.27 L	BIXAFEN (75g/l), PROTHIOCONAZOL (150g/l)

S2 Date of Fertilizer, fungicide, and weedkiller applications. Each treatment was applied equally on each plot.



S 3 Rarefaction curves of 16S rRNA genes sequences at (a) the top (0 to 5 cm) and (b) deeper (15 to 20 cm) soil indicating the observed number of operational taxonomic units (OTUs) at a genetic distance of 3% for the different tillage regime, i.e. conventional tillage (CT) vs. reduced tillage (RT), and crop residues management, i.e. residues retention (R+) vs. removal (R-).



S 4 Rarefaction curves of 28S rRNA genes sequences at (a) the top (0 to 5 cm) and (b) deeper (15 to 20 cm) soil indicating the observed number of operational taxonomic units (OTUs) at a genetic distance of 3% for the different tillage regime, i.e. conventional tillage (CT) vs. reduced tillage (RT), and crop residues management, i.e. residues retention (R+) vs. removal (R-).

Bacteria		
Taxon	**Indval**	**abund**
CT		
Acidimicrobiales	67.1	0.18
Actinobacteria	61.8	0.30
Actinocorallia	51.6	0.01
Aeromicrobium	40.5	0.03
Agromyces	53.8	0.05
Ardenticatenia	64.0	0.35
Arthrobacter	62.8	0.12
Bdellovibrionaceae	65.5	0.14
Betaproteobacteria	57.6	2.76
Blastopirellula	61.9	0.06
Burkholderiales	66.1	0.56
Caenimonas	63.2	0.09
Chlorobiales	59.1	0.43
Chthonomonadales	60.5	0.15
Elusimicrobia	58.5	0.29
Entotheonella	61.0	0.05
Gaiellales	73.4	0.11
Gammaproteobacteria	55.5	1.82
Gemmata	61.5	0.05
Gemmatimonadetes	68.0	0.10
Haloferula	51.3	0.02
Holophagae	59.6	1.43
Marinicella	61.4	0.14
Microlunatus	59.1	0.09
Mycobacterium	64.3	0.05
Nitrosomonadaceae	62.3	0.85
Nitrospirales	68.9	0.18
Nocardioides	61.8	0.20
Nordella	61.9	0.21
Pedobacter	61.8	0.18
Pontibacter	62.5	0.08
Rhizobiales	56.2	1.55
Rhodobacter	31.3	0.01
Rhodobium	57.4	0.30
Roseiflexus	56.3	0.03
Sandaracinaceae	61.5	0.13
Streptomyces	70.8	0.05
Thermomicrobia	46.3	0.02
Xanthomonadales	66.8	0.23
Zavarzinella	63.0	0.06
RT		
0-5 cm		
Acetobacteraceae	75.9	0.05
Acidovorax	55.7	0.03
Adhaeribacter	69.6	0.57
Albidiferax	60.2	0.07
Alphaproteobacteria	66.3	0.43
Altererythrobacter	66.6	0.15
Arenimonas	67.9	0.32
Armatimonadales	57.3	0.04
Armatimonadetes	61.2	0.82
Asticcacaulis	65.9	0.03
Bdellovibrio	67.5	0.28
Bosea	45.7	0.02
Bradyrhizobiaceae	64.2	0.84
Bryobacter	66.0	0.58
Caulobacter	74.8	0.10
Cellvibrio	66.1	0.11
Chitinophagaceae	56.0	3.29
Chthoniobacter	65.4	0.49
Comamonadaceae	65.9	0.87
Cyanobacteria	75.3	0.09
Cytophaga	85.3	0.10
Devosia	76.6	0.13
Dongia	59.5	0.05
Dyadobacter	76.1	0.16
Edaphobacter	35.0	0.01
Emticicia	60.7	0.02
Erythrobacteraceae	67.3	0.13
Ferruginibacter	70.3	1.18
Fibrobacteraceae	81.0	0.32
Flavisolibacter	64.5	0.77
Flavobacteriales	71.8	0.05
Flavobacterium	59.9	1.75
Fluviicola	56.7	0.06
Gemmatimonadacea	59.7	1.63
Gemmatimonas	66.7	0.13
Intrasporangiaceae	60.0	0.05
Legionella	56.0	0.03
Luteolibacter	79.6	0.25
Massilia	60.6	0.08
Mesorhizobium	66.2	0.25
Microbacteriaceae	56.3	0.07
Microcoleus	74.1	0.10
Microvirga	65.1	0.13
Mucilaginibacter	92.8	0.22
Myxococcales	64.7	0.36
Nitrosospira	67.6	0.82
Noviherbaspirillum	50.8	0.03
Novosphingobium	47.8	0.02
Opitutus	64.6	0.45
Oxalobacteraceae	72.0	0.37
Parasegetibacter	71.2	0.18
Pelomonas	91.7	0.06
Phenylobacterium	70.0	0.16
Pirellula	58.8	0.61
Pseudomonas	60.0	1.13
Pseudospirillum	70.4	0.05
Pseudoxanthomonas	55.0	0.02
Ramlibacter	72.6	0.13
Reyranella	70.2	0.56
Rhizobacter	60.2	0.09
Rhizobiaceae	78.2	0.15
Rhizomicrobium	87.3	0.23
Rhodanobacter	76.4	0.07
Rickettsiales	70.7	0.14
Saprospiraceae	63.0	1.04
Schlesneria	64.9	0.05
Sediminibacterium	52.6	0.02
Segetibacter	66.9	0.05
Skermanella	59.0	0.06
Sphingobacteriaceae	40.6	0.02
Sphingobacteriales	65.9	1.68
Sphingobium	39.3	0.02
Sphingomonadaceae	77.5	0.17
Sphingomonadales	60.5	0.44
Sphingomonas	63.1	1.64
Taibaiella	79.5	0.14
Thermomonas	66.3	0.09
Vampirovibrionales	82.9	0.09
Variovorax	61.3	0.16
Verrucomicrobiaceae	61.7	0.10
Xanthomonadaceae	66.3	0.09
15-20 cm		
Acidobacteria	58.9	14.73
Anaerolineaceae	62.8	7.45
Bacillus	68.3	0.25
Chloroflexi	57.2	1.35
Chryseolinea	57.3	1.24
Cytophagales	60.2	0.37
Deltaproteobacteria	65.7	3.36
Desulfuromonadales	60.5	0.05
Ignavibacteriales	73.7	0.22
Nitrospira	57.8	5.07
Planctomycetes	62.7	0.17
Solirubrobacterales	60.4	0.09
CT, 0-5 cm		
Aquabacterium	43.3	0.02
Bacteriovorax	39.8	0.01
Cellulomonas	41.7	0.01
Defluviimonas	40.0	0.01
Fibrella	37.5	0.004
Filimonas	38.9	0.01
Hyphomicrobiaceae	36.8	0.07
Kribbella	44.4	0.01
Lacibacter	62.2	0.05
Leptolyngbya	37.5	0.01
Paucimonas	44.1	0.02
Phycisphaerae	33.3	0.62
Plantibacter	37.5	0.004
Polaromonas	36.5	0.19
Prosthecobacter	51.1	0.01
Pseudonocardiaceae	59.2	0.06
Rhodococcus	41.7	0.02
Rhodospirillales	36.1	0.68
Simiduia	37.5	0.005
Sorangium	46.9	0.01
Verrucomicrobiales	41.7	0.01
RT, 0-5 cm		
Aquicella	33.2	0.48
Rhodobacteraceae	52.1	0.01
Roseomonas	40.5	0.05
CT, 15-20 cm		
RT, 15-20 cm		
Paenibacillus	55.2	0.08
Rhodocyclaceae	39.1	0.08
Ureibacillus	50.0	0.005

S 5 Indicator bacterial taxa found for each set of soil conditions, their relative abundance (abund, %) and their indicative value (Indval, %). *CT=conventional tillage and RT=reduced tillage.*

Fungi		
Taxon	**Indval**	**abund**
CT		
Atractiella	69.5	0.27
Bionectriaceae	50.9	0.06
Cercophora	69.5	6.66
Chytridiales	61.3	0.12
Cryptococcus	61.6	0.08
Cudoniella	69.2	0.06
Galactomyces	56.5	0.03
Kriegeria	60.8	0.34
Lasionectria	63.0	0.23
Lasiosphaeriaceae	72.0	1.01
Pyrenochaeta	72.4	0.77
Saccharomycetales	76.4	0.21
Schizothecium	58.7	2.70
Xylariales	59.6	2.39
RT		
Ceratobasidiaceae	83.1	4.32
Lycoperdon	31.3	0.01
Massariosphaeria	31.3	0.01
Nectria	63.2	0.25
0-5 cm		
Chytridiomycetes	69.4	2.10
Dothideomycetes	65.5	0.07
Gaeumannomyces	67.1	0.27
Gibberella	69.2	1.69
Pleosporales	58.7	0.99
Rhizophydiales	65.9	0.14
Saccharomycetaceae	66.6	0.10
Spizellomyces	72.3	0.11
Tremellaceae	60.8	0.10
15-20 cm		
Aniptodera	63.9	0.14
Ascomycota	65.4	1.32
Capnodiales	67.4	0.21
Chaetomiaceae	61.6	0.94
Clavicipitaceae	66.3	0.42
Didymellaceae	58.9	1.37
Diversisporales	60.7	0.09
Doratomyces	45.0	0.03
Glomus	81.8	0.47
Guehomyces	62.7	4.52
Herpotrichiellaceae	56.4	1.71
Hypocreales	63.6	2.63
Leotiomycetes	73.7	2.53
Melastiza	64.3	0.06
Microascaceae	69.6	0.33
Mrakia	73.7	0.27
Paecilomyces	65.6	0.43
Penicillium	71.7	0.16
Plectosphaerella	61.9	4.69
Pyronemataceae	64.4	7.48
Sordariomycetes	68.3	5.04
Tetracladium	59.7	6.69
Tremellomycetes	67.3	3.04
Trichocomaceae	65.2	0.20
CT, 0-5 cm		
Clavulinaceae	42.9	0.07
Cyphellophora	37.5	0.01
Pluteaceae	46.3	0.03
Pyxidiophora	50.0	0.15
Tricholomataceae	79.9	0.43
RT, 0-5 cm		
Bionectria	43.2	0.13
Tubaria	55.1	0.08
CT, 15-20 cm		
RT, 15-20 cm		
Pulvinula	84.2	0.15
Waitea	38.0	0.06

S 6 Indicator fungal taxa found for each set of soil conditions, their relative abundance (abund, %) and their indicative value (Indval, %). *CT=conventional tillage and RT=reduced tillage.*

8. References

Bahram, M., Peay, K.G., Tedersoo, L., 2015. Local-scale biogeography and spatiotemporal variability in communities of mycorrhizal fungi. New Phytol. 205, 1454–1463. doi:10.1111/nph.13206

Baker, G.C., Smith, J.J., Cowan, D.A., 2003. Review and re-analysis of domain-specific 16S primers. J. Microbiol. Methods 55, 541–555. doi:10.1016/j.mimet.2003.08.009

Bardgett, R.D., 2002. Causes and consequences of biological diversity in soil1. Zoology 105, 367–375. doi:10.1078/0944-2006-00072

Baselga, A., 2010. Partitioning the turnover and nestedness components of beta diversity. Glob. Ecol. Biogeogr. 19, 134–143. doi:10.1111/j.1466-8238.2009.00490.x

Bottalico, A., Perrone, G., 2002. Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur. J. Plant Pathol. 108, 611–624.

Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen—total. Am. Soc. Agron., Methods of soil analysis 595–624.

Cardenas, E., Tiedje, J.M., 2008. New tools for discovering and characterizing microbial diversity. Curr. Opin. Biotechnol. 19, 544–9. doi:10.1016/j.copbio.2008.10.010

Ceja-Navarro, J.A., Rivera-Orduña, F.N., Patiño-Zúñiga, L., Vila-Sanjurjo, A., Crossa, J., Govaerts, B., Dendooven, L., 2010. Phylogenetic and Multivariate Analyses To Determine the Effects of Different Tillage and Residue Management Practices on Soil Bacterial Communities. Appl. Environ. Microbiol. 76, 3685–3691. doi:10.1128/AEM.02726-09

Clapperton, M.J., 2003. Increasing soil biodiversity through conservation agriculture: managing the soil as a habitat. Proc Second World Cong Conserv. Agric. Prod. Harmony Nat. Iguassu Falls Parana-Braz. 11–15 August 2003.

Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Marsh, T., Garrity, G.M., Tiedje, J.M., 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 37, D141–D145. doi:10.1093/nar/gkn879

Colwell, R.K., 2005. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. Persistent URL: purl.ococ.org/estimates 2013.

Degrune, F., Dufrêne, M., Colinet, G., Massart, S., Taminiau, B., Bodson, B., Hiel, M.-P., Daube, G., Nezer, C., Vandenbol, M., 2015. A novel sub-phylum method discriminates better the impact of crop management on soil microbial community. Agron. Sustain. Dev. 35, 1157–1166. doi:10.1007/s13593-015-0291-4

Denslow, J.S., Pickett, S.T.A., White, P.S., 1985. Disturbance-mediated coexistence of species, The Ecology of Natural Disturbance and Patch Dynamics.

Dorr de Quadros, P., Zhalnina, K., Davis-Richardson, A., Fagen, J.R., Drew, J., Bayer, C., Camargo, F.A., Triplett, E.W., 2012. The effect of tillage system and crop rotation on soil microbial diversity and composition in a subtropical Acrisol. Diversity 4, 375–395. doi:10.3390/d4040375

Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species:the need for a flexible asymmetrical approach. Ecol. Monogr. 67, 345–366. doi:10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.

Eilers, K.G., Debenport, S., Anderson, S., Fierer, N., 2012. Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biol. Biochem. 50, 58–65. doi:10.1016/j.soilbio.2012.03.011

Fierer, N., Ladau, J., Clemente, J.C., Leff, J.W., Owens, S.M., Pollard, K.S., Knight, R., Gilbert, J.A., McCulley, R.L., 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. Science 342, 621–624. doi:10.1126/science.1243768

Francis, C.A., Beman, J.M., Kuypers, M.M.M., 2007. New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. ISME J. 1, 19–27. doi:10.1038/ismej.2007.8

Gage, D.J., 2004. Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. Microbiol. Mol. Biol. Rev. 68, 280–300. doi:10.1128/MMBR.68.2.280-300.2004

Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41, 109–117. doi:10.1139/m95-015

Govindasamy, V., Senthilkumar, M., Magheshwaran, V., Kumar, U., Bose, P., Sharma, V., Annapurna, K., 2011. Bacillus and Paenibacillus spp.: potential PGPR for sustainable agriculture, in: Plant Growth and Health Promoting Bacteria. Springer, pp. 333–364.

Hobbs, P.R., Sayre, K., Gupta, R., 2008. The role of conservation agriculture in sustainable agriculture. Philos. Trans. R. Soc. B Biol. Sci. 363, 543–555. doi:10.1098/rstb.2007.2169

Kibblewhite, M.G., Ritz, K., Swift, M.J., 2008. Soil health in agricultural systems. Philos. Trans. R. Soc. B Biol. Sci. 363, 685–701.

Kladivko, E.J., 2001. Tillage systems and soil ecology. Soil Tillage Res. 61, 61–76. doi:10.1016/S0167-1987(01)00179-9

Knops, J.M.H., Tilman, D., 2000. Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. Ecology 81, 88–98.

Koorem, K., Gazol, A., Öpik, M., Moora, M., Saks, Ü., Uibopuu, A., Sõber, V., Zobel, M., 2014. Soil Nutrient Content Influences the Abundance of Soil Microbes but Not Plant Biomass at the Small-Scale. PLoS ONE 9, e91998. doi:10.1371/journal.pone.0091998

Kurtzman, C.P., Robnett, C.J., 1997. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5'end of the large-subunit (26S) ribosomal DNA gene. J. Clin. Microbiol. 35, 1216–1223.

Lienhard, P., Terrat, S., Prévost-Bouré, N.C., Nowak, V., Régnier, T., Sayphoummie, S., Panyasiri, K., Tivet, F., Mathieu, O., Levêque, J., Maron, P.-A., Ranjard, L., 2013. Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland. Agron. Sustain. Dev. 34, 525–533. doi:10.1007/s13593-013-0162-9

Loreau, M., 2004. Does functional redundancy exist? Oikos 104, 606–611. doi:10.1111/j.0030-1299.2004.12685.x

Marhan, S., Philippot, L., Bru, D., Rudolph, S., Franzaring, J., Högy, P., Fangmeier, A., Kandeler, E., 2011. Abundance and activity of nitrate reducers in an arable soil are more affected by temporal variation and soil depth than by elevated atmospheric [CO2]. FEMS Microbiol. Ecol. 76, 209–219. doi:10.1111/j.1574-6941.2011.01048.x

Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670. doi:10.1046/j.1351-0754.2003.0556.x

Nannipieri, P., Badalucco, L., 2003. Biological processes, in: Handbook of Processes and Modelling in the Soil-Plant System. Benbi, D.K., Nieder, R., Binghamton, NY, pp. 57–82.

Navarro-Noya, Y.E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M.C., Valenzuela-Encinas, C., Jiménez-Bueno, N., Verhulst, N., Govaerts, B., Dendooven, L., 2013. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biol. Biochem. 65, 86–95. doi:10.1016/j.soilbio.2013.05.009

Nelson, D.W., Sommers, L.E., 1982. Total Carbon, Organic Carbon, and Organic Matter. Methods Soil Anal. Part 2 Chem. Microbiol. Prop. Agronomy Monograph, 539–579.

Nilsson, L.O., Bååth, E., Falkengren-Grerup, U., Wallander, H., 2007. Growth of ectomycorrhizal mycelia and composition of soil microbial communities in oak forest soils along a nitrogen deposition gradient. Oecologia 153, 375–384.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J., Suggests, M., 2007. The vegan package. Community Ecol. Package 631–637.

Olsson, P.A., Baath, E., Jakobsen, I., 1997. Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of Fatty Acid signatures. Appl. Environ. Microbiol. 63, 3531–3538.

Paustian, K., Six, J., Elliott, E.T., Hunt, H.W., 2000. Management options for reducing CO2 emissions from agricultural soils. Biogeochemistry 48, 147–163.

Poisot, T., Péquin, B., Gravel, D., 2013. High-Throughput Sequencing: A Roadmap Toward Community Ecology. Ecol. Evol. 3, 1125–1139. doi:10.1002/ece3.508

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35, 7188–7196. doi:10.1093/nar/gkm864

R Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rahman, M.H., Okubo, A., Sugiyama, S., Mayland, H.F., 2008. Physical, chemical and microbiological properties of an Andisol as related to land use and tillage practice. Soil Tillage Res. 101, 10–19. doi:10.1016/j.still.2008.05.006

Rhoton, F.E., 2000. Influence of Time on Soil Response to No-Till Practices. Soil Sci. Soc. Am. J. 64, 700–709. doi:10.2136/sssaj2000.642700x

Schimel, J., 1995. Ecosystem consequences of microbial diversity and community structure, in: Chapin, F.S., Koerner, C. (Editors). Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences. Springer, pp. 239–254.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Horn, D.J.V., Weber, C.F., 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl. Environ. Microbiol. 75, 7537–7541. doi:10.1128/AEM.01541-09

Scopel, E., Triomphe, B., Affholder, F., Silva, F.A.M.D., Corbeels, M., Xavier, J.H.V., Lahmar, R., Recous, S., Bernoux, M., Blanchart, E., Mendes, I. de C., Tourdonnet, S.D., 2012. Conservation agriculture cropping systems in temperate and tropical conditions, performances and impacts. A review. Agron. Sustain. Dev. 33, 113–130. doi:10.1007/s13593-012-0106-9

Sengupta, A., Dick, W.A., 2015. Bacterial Community Diversity in Soil Under two Tillage Practices as Determined by Pyrosequencing. Microb. Ecol. 70, 853–859. doi:10.1007/s00248-015-0609-4

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Tilman, D., 1982. Resource Competition and Community Structure. Princeton University Press Princeton, NJ.

Chapter V. Tillage effect over the growing season

Temporal dynamics of microbial communities under two contrasting tillage regimes

In preparation, to be submitted in *Frontiers in Microbiology*.

Florine Degrune, Nicolas Theodorakopoulos, Gilles Colinet, Bernard Bodson, Marie-Pierre Hiel, Bernard Taminiau, Carine Nezer, Georges Daube, Micheline Vandenbol, Martin Hartmann

Outline

The aim of the work was to explore the α- and β-diversity responses associated with the tillage regime and crop residue management practice over the growing season of two crops: *Vicia faba* and *Triticum aestivum*. Here we employed an innovative data visualization method to display the entire taxonomic diversity of microorganisms and associated soil management microbial patterns.

1. Abstract

Agricultural productivity relies on a wide range of ecosystem services provided by the soil biota. Plowing is a fundamental component of conventional farming, but long-term detrimental effects such as soil erosion and loss of soil organic matter have been recognized. Moving towards more sustainable management practices such as reduced tillage or crop residue retention can reduce these detrimental effects, but will also influence structure and function of the soil microbiota with direct consequences for the associated ecosystem services. Although there is increasing evidence that different tillage regimes alter the soil microbiome, we have a limited understanding of the temporal dynamics of these effects. Among the few studies that explored this question, none of them harnessed the power of metabarcoding techniques to infer structural shifts in the microbiome. Here, we used high-throughput sequencing of bacterial and fungal ribosomal markers to explore changes in soil microbial community structure under two contrasting tillage regimes (conventional and reduced tillage) either with or without crop residue retention over different growing stages of two crops (*Vicia faba* and *Triticum aestivum*). Tillage and growing stage were significant determinants of microbial community structure, but the impact of tillage showed only moderate temporal dependency. Whereas the tillage effect on soil bacteria showed some temporal dependency and became less strong at later growing stages, the tillage effect on soil fungi was more consistent over time. Crop residue retention had only a minor influence on the community. Six years after the conversion from conventional to reduced tillage, soil moisture contents and nutrient levels were significantly lower under reduced than under conventional tillage. These changes in edaphic properties were related to specific shifts in microbial community structure. Notably, bacterial groups featuring copiotrophic lifestyles or potentially carrying the ability to degrade more recalcitrant compounds were favoured under conventional tillage, whereas taxa featuring more oligotrophic lifestyles were more abundant under reduced tillage. Our study found that, under the specific edaphic and climatic context of central Belgium, different tillage regimes created different ecological niches that select for different microbial lifestyles with potential consequences for the ecosystem services provided to the plants and their environment.

2. Introduction

It is well recognized that agricultural productivity strongly relies on a wide range of ecosystem services provided by the soil biota (Altieri, 1999). Although the delivery of ecosystem services are driven by complex interactions between the soil biota and abiotic parameters (Kibblewhite et al., 2008), most soil processes related to organic matter transformation and nutrient cycling are mediated by microorganisms (Nannipieri and Badalucco, 2003). Moreover, some specific symbiotic groups such as plant-growth promoting rhizobacteria and mycorrhizal fungi are well known to favor crop productivity and plant health by stimulating plant growth and protecting plants against pathogens (Berg, 2009; Siddiqui et al., 2008). Microorganisms also contribute to soil aggregate formation and aeration, as well as carbon sequestration in agroecosystems (Six et al., 2006).

Plowing is one of the main components of conventional farming and has been used for centuries to control weeds, prepare the seedbed, temporary alleviate soil compaction, suppress soil-borne diseases, and improve nutrient mineralization and availability (Hobbs et al., 2008). Besides these short-term benefits, long-term detrimental effects such as soil erosion and loss of soil organic matter have been recognized (Montgomery, 2007; Six et al., 1999). Alternative soil management practices such as reduced or zero tillage, crop residue retention, mulching, crop rotation, and intercropping can significantly enhance both soil quality and crop productivity in agroecosystems (Scopel et al., 2012).

Moving towards more sustainable agricultural management and more specifically towards reduced tillage with crop residue retention is not without consequences for the soil microbiota in terms of structure (α- and β-diversity) and function. Several studies have reported effects of soil tillage and/or crop residue management on soil microbial community structures (Carbonetto et al., 2014; Degrune et al., 2016; Dorr de Quadros et al., 2012; Jiménez-Bueno et al., 2016; Navarro-Noya et al., 2013; Sengupta and Dick, 2015). For example, the diversity of arbuscular mycorrhizal fungi (AMF), a group of fungi supporting the host plant with enhanced nutrient acquisition and increased resistance against drought and root pathogens (Van der Heijden et al., 1998), has shown to be increased under reduced tillage (Säle et al., 2015). Other studies have shown that enzymatic activities related to soil organic C, N, P, and S cycling increased when applying principles of conservation agriculture such as zero-tillage and/or crop residue retention (Murphy et al., 2016;

Panettieri et al., 2014). Residue retention also appeared to increase soil microbial biomass (Salinas-Garcia et al., 2001).

In general, the soil microbiota is affected by various abiotic factors such as pH (Lauber et al., 2009), soil moisture (Brockett et al., 2012), oxygen availability (Lüdemann et al., 2000), quality of organic substrates (Bending et al., 2002), nutrient inputs such as nitrogen and phosphorus (Leff et al., 2015), soil type (Chau et al., 2011; Girvan et al., 2003) and temperature (Frey et al., 2008), as well as biotic factors such as plant communities (Kowalchuk et al., 2002) and the occurrence of other soil organisms such as earthworms (Héry et al., 2007). Many of these parameters are likely to change with tillage regime and crop residue management, which in return may influence soil microbial communities and the ecosystem services they provide.

In addition to the tillage regime and crop residue management, growing stage of the crop is a major driver of microbial community structure in agricultural systems (Houlden et al., 2008; Lauber et al., 2013). Root system development over the growing stage and associated changes in rhizodeposition may alter the spatial distribution and quality of organic materials (Philippot et al., 2013), influencing the dynamics of the microbial community over time. Although previous studies have investigated the growing stage effect on microbial community structure, only few have looked at the dynamics under different soil treatments over the course of a growing season (Shi et al., 2013; Spedding et al., 2004; Zhang et al., 2012). These previous studies did not harness the power of the emerging high-throughput sequencing technologies in order to assess such effects at a higher coverage and taxonomic resolution. Since individual members of the soil microbiota can have both beneficial and detrimental effects on crop growth and productivity, a detailed assessment of their specific response is of primary interest.

In the presented study, three different hypotheses have been tested: (1) Different tillage regimes favour different microbial communities by altering soil physical and chemical properties, (2) tillage effects vary across the growing stages and differences in community structure between conventional and reduced tillage get smaller towards the end of the growing season, and (3) tillage-induced changes in soil physicochemical properties including moisture, aeration, and nutrient availability favor different microbial life strategies. To test these hypotheses, we employed a 454 pyrosequencing approach of bacterial and fungal ribosomal markers to examine the response of soil microbial community structure to 6 years of continuous reduced and conventional tillage combined with residue retention or removal over the course of different growing stages of two

crops, i.e. *Vicia faba* (faba bean) and *Triticum aestivum* (wheat), in an experimental field located in central Belgium and characterized by a loess-derived soil. Understanding the microbial taxon-level response over the growing season in soils subjected to different management practices has the potential to optimize current agricultural practices in order to promote beneficial microorganisms and, thus, improve the sustainability of agriculture.

3. Materials and methods

Site description

The SOLRESIDUS long-term experiment, located on the experimental farm of Gembloux Agro-Bio Tech (University of Liège, Belgium, at 50°33'45.92"N and 4°42'48.97"E), is characterized by an oceanic temperate climate and a Cutanic Luvisol. The soil texture is silt loam and largely dominated by silt (70-80%), clay (18-22%) and sand (5-10%). The monthly average temperature is highest in July, at 18.4°C, and lowest in January, at 3.3°C. The monthly average rainfall is highest in December, at 81 mm, and lowest in April, at 51.3 mm (data from the Belgian Royal Meteorological Institute).

Soil treatments and experimental design

The experimental design consisted of a Latin square arrangement with four replicates of four soil treatments and has previously been described in detail (Degrune et al., 2016). Briefly, each soil treatment consisted of a combination of different soil practices: a tillage practice (conventional or reduced tillage) combined with a crop residue management practice (residue retention or removal). The combinations were as follows: conventional tillage with residue removal (CT/-R, the agricultural practice most commonly used in Belgium), conventional tillage with residue retention (CT/+R), reduced tillage with residue removal (RT/-R), and reduced tillage with residue retention (RT/+R). Conventionally tilled plots were plowed to a depth of 25 cm, while in the plots under reduced tillage only the top 10 cm of the soil was mixed (shallow tillage). Crops were rotated on the studied field and crop history is as follows: Brassica napus (2009), Triticum aestivum (2010, 2011 and 2012), *Vicia faba* (2013), and *Triticum aestivum* (2014).

Soil sampling and soil chemical analysis

Soil samples were collected from each of the 16 plots in 2013 (*Vicia faba*) and 2014 (*Triticum aestivum*) at different growing stages including the seedling, leaf development and flowering stages for *Vicia faba*, as well as tillering and grain filling stages for *Triticum aestivum*. Each soil sample corresponded to a composite of six randomly selected soil cores of 5 cm length and 2 cm diameter each and collected at a depth of 15 cm with an auger. In RT and at a depth of 15 cm, the soil was undisturbed for 6 years. The detail of field operations is provided in **Table 9**.

Table 9 Field operations performed on the SOLRESIDUS experiment in 2012 and 2013.

Date	Operation field	Plot	Date	Operation field	Plot	Date	Operation field	Plot
2012			**2013**			**2014**		
29/08	Shallow tillage	All	18/03	Weeding	All	11/03	Nitrogen fertilization	All
06/09	Cover crop sowing (mustard)	All	05/04	Sowing faba bean	All	26/03	Soil sampling	All
13/12	plowing	CT	08/04	Meadow-emergence weeding	All	01/04	Weeding	All
			15/04	Soil sampling	All	15/04	Nitrogen fertilization	All
			24/05	Soil sampling	All	15/04	Growth regulator	All
			27/06	Soil sampling	All	25/04	Weeding	All
			08/07	Chemical pest control	All	27/04	Fungicide	All
			28/08	Weeding	All	12/05	Nitrogen fertilization	All
			04/09	Faba bean harvest	All	16/05	Weeding	All
			25/11	Plowing	CT	26/05	Soil sampling	All
			25/11	Shallow tillage	All	06/06	Fungicide	All
			25/11	Sowing winter wheat	All	04/09	Winter wheat harvest	All

Soil physical and chemical properties of each sample were determined as outlined in the following. Water content was measured by drying soil samples at 105°C during 48h. Soil pH was measured in 1M KCl (2:5 w:v) after two hours of equilibration. Water-extractable elements were quantified by flame absorption (Ca, Mg), flame emission (P, Na), or colorimetry (P) after extraction of 20 g of 8-mm-sieved fresh soil in 100 ml H2O for 1 h at room temperature and filtration on 602 H 1/2. Hot water carbon was quantified as described by Ghani et al. (2003). Nitrates (NO3) and ammonium

(NH4+) were determined in 2M KCl of soil extracts by flow injection analysis, using QuickChem® (Method 12-107-06-3-B, Lachat instruments 5600 lindburgh drive Loveland, CO 80539 USA).

Pyrosequencing of 16S and 28S rRNA genes

DNA extraction and pyrosequencing of bacterial and fungal ribosomal markers were fully described by Degrune et al. (2016). Briefly, the V1-V3 region of the 16S rRNA gene (approx. 500 bp) and the D1-D2 region of the 28S rRNA gene (approx. 700 bp) were unidirectionally sequenced using the GS junior-FLX Titanium technology (Roche 454 Life Sciences, Brandford, CT, USA). Sequence data were processed according to Hartmann et al. (2014) including procedures to reduce the influence of sequencing errors (Quince et al., 2009), PCR substitution errors (Quince et al., 2009), and chimeras (Edgar et al., 2011) as implemented in mothur (Schloss, 2009), as well as target verification and extraction (Hartmann et al., 2010; Nilsson et al., 2010). Denoised sequences were clustered into operational taxonomic units (OTUs) using CROP (Hao et al., 2011) at 97% sequence identity. CROP center sequences were queried against SILVA (16S rRNA) and RDP (28S rRNA) (Maidak et al., 1996; Quast et al., 2012) using the naive Bayesian classifier (Wang et al., 2007) implemented in mothur and a minimum bootstrap support of 60%.

Statistics and data visualization

All statistical analyses were performed using Primer6+ (Clarke and Gorley, 2006) and the R software (R Development Core Team, 2011). Adjustments for multiple testing were performed using the false discovery rate correction according to Storey (2002) performed with qvality (Käll et al., 2009) unless indicated otherwise. Differences in β-diversity were examined using the Bray-Curtis ecological distance calculated from normalized and square-root transformed OTU abundances. The significance of the experimental factors was tested using multivariate permutational analysis of variance (PERMANOVA, Anderson, (2001)) as implemented in Primer6+ with 99,999 permutations. The heterogeneity of variance between groups was tested using permutational analysis of dispersion (PERMDISP, Anderson, (2001)) as implemented in Primer6+ with 99,999 permutations. The major variance components of bacterial and fungal β-diversity were visualized using principal coordinate analyses (PCO, Gower (1966)). Estimates of α-diversity, i.e. observed richness Sobs and Smith-Wilson evenness E (Smith and Wilson, 1996), were based on evenly rarefied OTU abundance matrices using an iterative subsampling procedure with 1000 iterations as implemented in mothur. The significance of the experimental factors on α-diversity

and soil physical and chemical parameters were examined using univariate PERMANOVA based on Euclidean distances calculated from z-transformed data as implemented in Primer6+ with 99,999 permutations. The relationship between the soil properties and microbial community structure was assessed using the distance-based linear modeling (DistLM, McArdle & Anderson (2001)) procedure implemented in Primer6+ with 99,999 permutations.

The response of individual taxa at high (phylum) and low (OTUs) resolution was evaluated using PERMANOVA as implemented in the adonis function of the R package vegan (Oksanen et al., 2007). In order to visualize positive or negative responses of the individual taxa to one of the tillage regimes, the relative abundances were z-transformed and then averaged by tillage. The same analysis was performed on the individual soil physico-chemical parameters. Taxonomic networks were used to visualize the OTU distribution across the taxonomic hierarchy (Frey et al., 2016; Hartmann et al., 2015). The response of the significant OTUs to tillage was represented by values derived from z-transformed data independent from the growing stages (i.e. centered by stage), and ranged from -1 to 1. The network was generated in Cytoscape 3.3.0 (Shannon et al., 2003) using the Allegro Fruchterman-Reingold algorithm (Fruchterman and Reingold, 1991). The network is characterized by nodes (= OTUs) and edges (= taxonomic path from phylum to OTU level), whereas OTUs are placed at the level of the lowest possible taxonomic assignment. The response of individual OTUs to tillage was mapped onto the taxonomic network. All OTUs are visualized but significant responses were only evaluated for the robust OTUs occurring in at least 25% of all samples (i.e. 20 samples).

5. Results

Effect of soil management and growing season on β- and α-diversity

A total of 393,004 (4,913±1,887 per sample) bacterial 16SV2-V3 and 456,709 (5,709±, 1312 per sample) fungal 28SD1 high-quality sequences were obtained for the 80 soil samples, yielding a total of 1710 bacterial and 1567 fungal OTUs. Growing season and tillage regime emerged as important factors driving microbial β-diversity, explaining 23-27% and 20-21% of the variance, respectively (**Table 10**). Management of the crop residues showed no (bacteria) or only small (fungi) influence on β-diversity (**Table 10**). These shifts in bacterial and fungal β-diversity due to tillage and growing stage became evident in the PCO plots, with communities clustering by tillage regime on the first (bacteria) or second (fungi) axis (**Figure 26a**). Compositional shifts due to crop and growing stage became evident on the corresponding other main component, e.g. the fungal communities of growing stages 2 and 3 being strongly separated from the other stages.

Around 6% of the variance in bacterial and fungal β-diversity was explained by an interaction between tillage and growing season (**Table 10**). This interaction became evident when examining the pairwise tests (**Table 10**). For faba bean, the bacterial and fungal communities showed the highest dissimilarity between CT and RT at stage 2, and the communities became again more similar at stage 3. For wheat, the bacterial communities were different between CT and RT at stage 4 but not at stage 5, whereas the fungal communities were distinct at both growing stages.

Bacterial α-diversity was mainly influenced by tillage regime, while fungal α-diversity was mostly influenced by the growing stage (**Table 11 & Figure 26b**). For bacteria, CT was more rich and less even than RT, while for fungi, richness remained similar between CT and RT, and CT was less even.

As differences in α- and β-diversity between CT and RT can arise from differences in similarity, differences in dispersion or both, a separate test of dispersion using PERMDISP was used to detect the nature of such differences. Results reported no differences in dispersion, i.e. homogeneity of variance, suggesting that differences in α- and β-diversity were largely driven by dissimilarity rather than dispersion (see PERMDISP on **Figure 26**).

Table 10 Effects of tillage regime, crop residue management, and growing season on bacterial and fungal β-diversity.

	Bacteria			*Fungi*		
Main test[a]	**F**	**P (perm)**	**VC**	**F**	**P (perm)**	**VC**
Tillage	6.7	0.00001°	20	11	0.00001°	21
Residue	0.9	0.61	Neg	1.4	0.013°	4
Stage	4.0	0.00001°	23	8.2	0.00001°	27
Tillage*stage	1.1	0.08	6	1.2	0.008°	6
Pairwise test[b]	**t**	**Padjust**	**Avg sim**	**t**	**Padjust**	**Avg sim**
1CT, 1RT	1.6	0.0007°	69.9	1.8	0.0004°	59.9
2CT, 2RT	1.6	0.0007°	67.0	2.1	0.0003°	51.7
3CT, 3RT	1.3	0.035°	73.3	1.5	0.007°	58.2
4CT, 4RT	2.0	0.0007°	71.3	1.9	0.0003°	56.4
5CT, 5RT	1.1	0.2	69.3	1.6	0.0003°	58.7

Effects of main factors and their interactions as assessed by multivariate permutational analysis of variance (PERMANOVA). Main factors represent tillage (CT, RT), residue management (R+, R-), and growing stage (1=seedling, 2=leaf development, 3=flowering, 4=tillering, 5=grain filling). *Vicia faba* correspond to stages 1, 2 and 3, and *Triticum aestivum* correspond to stages 4 and 5. Values represent the pseudo-F ratio[2] (F), the permutation-based level of significance (P(perm)) and the estimation of the variance component (VC). (b) Pairwise comparisons between tillage regimes for each growing stage. Values represent the univariate t-statistic (t), the permutation-based level of significance adjusted for multiple comparisons using false discovery rate correction according to Benjamini-Hochberg (Benjamini and Hochberg, 1995) (Padjust), and the average between-group Bray-Curtis similarity (avg sim).

Table 11 Effects of tillage regime, crop residue management, and growing season on bacterial and fungal α-diversity.

	Bacteria		*Fungi*	
Maint test[a]	***Richness (sobs)***	***Evenness (sw)***	***Richness (sobs)***	***Evenness (sw)***
	F(P)	**F(P)**	**F(P)**	**F(P)**
Tillage	36.3 (0.00001°)	26.1 (0.00001°)	3.6 (0.06)	16.6 (0.0002°)
Residue	0.08 (0.8)	0.8 (0.4)	0.5 (0.5)	2.2 (0.1)
Stage	4.1 (0.005°)	1.2 (0.3)	10.7 (0.00001°)	43.9 (0.00001°)
Tillage*stage	0.47 (0.8)	0.6 (0.7)	2.1 (0.09)	1.1 (0.4)

(a) Effects of main factors and their interactions as assessed by univariate permutational analysis of variance (PERMANOVA). Main factors represent tillage (CT, RT), residue management (R+, R-), and growing stage (1=seedling, 2=leaf development, 3=flowering, 4=tillering, 5=grain filling). *Vicia faba* correspond to stages 1, 2 and 3, and *Triticum aestivum* correspond to stages 4 and 5. Values represent the pseudo-F ratio (F) and the permutation-based level of significance (P).

[2] The pseudo-F ratio derived from the Fisher's traditional F -ratio, but it is associated with permutation test in multivariate analysis to partition the variance among distance matrices (Anderson and Braak, 2003).

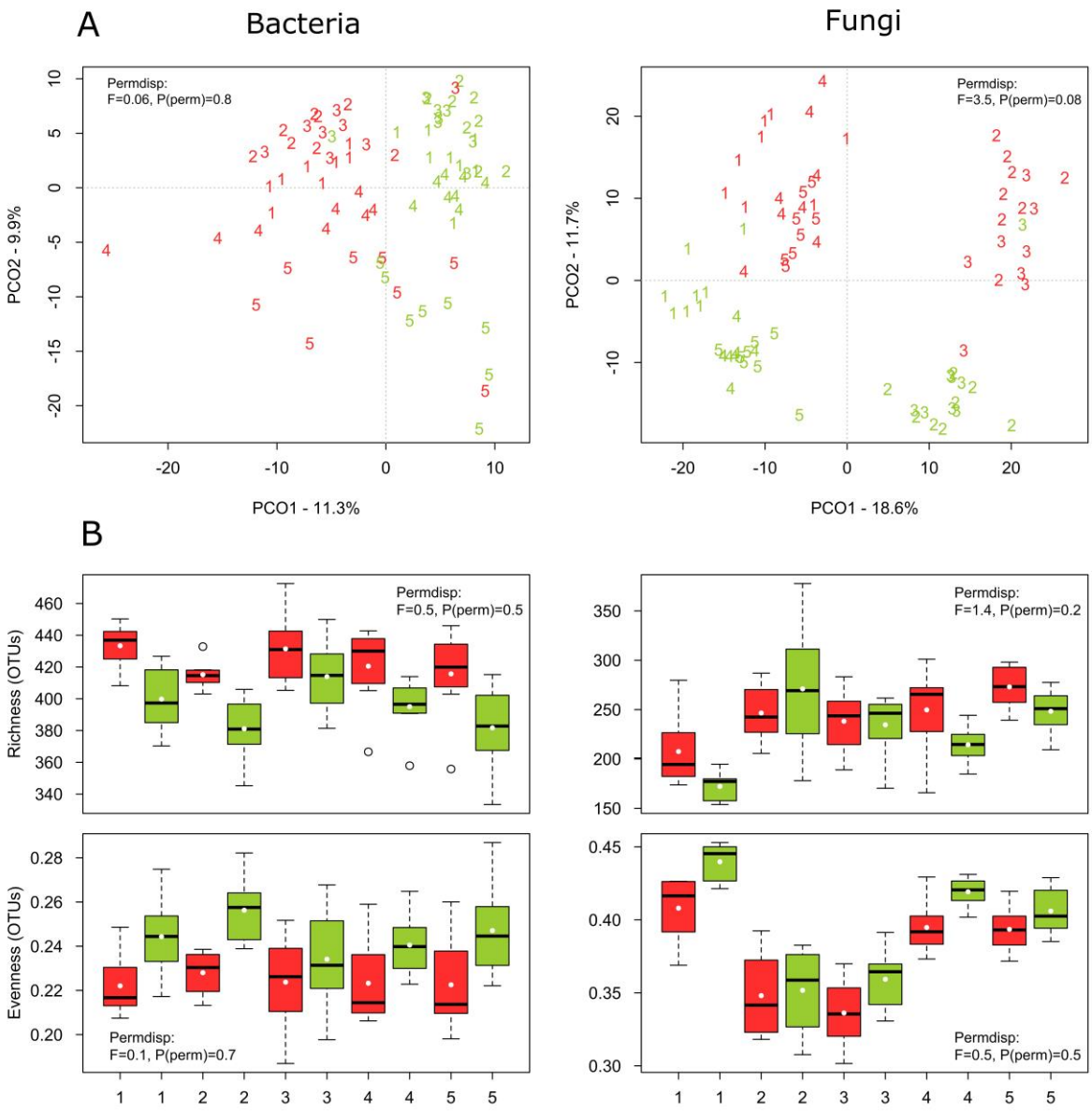


Figure 26 Effects of tillage regime and growing stage on bacterial and fungal β- (A) and α-diversity (B). The PCO ordination axes PCO1 and PCO2 explain 11% and 10% of the bacterial community variation, respectively, and 19% and 12% of the fungal community variation, respectively. Tillage regime is represented by color code with CT=red and RT=red and growing season is represented by numbers with 1=seedling, 2=leaf development, 3=flowering, 4=tillering, 5=grain filling). The faba bean season corresponds to stages 1, 2 and 3, and the wheat season corresponds to stages 4 and 5. Values represent the heterogeneity of variance for tillage effect as assessed by PERMDISP.

Relationship between soil chemical properties and microbial β-diversity

Our findings identified Growing stage as the main driver of overall soil chemical properties (F=20, p=0.00001) and tillage regime as the second (F=6, p=0.00003), while no crop residue effect (F=0.9, p=0.5) was observed. A low but significant interaction effect between growing stage and tillage regime was noticed (F=1.6, p=0.03). Based on the main test provided for each soil parameter (**Table 12a**), we identified the levels of P, K, Ca, NO3, Nmin and soil moisture to change with tillage regime. CT consistently showed higher levels of these properties when compared to RT (**Figure 27a**). Several parameters also revealed an interaction effect between the tillage and the growing stage (**Table 12a & Figure 27b**), indicating significant variability in the tillage effect across the growing season.

The relationship between microbial community structure and soil chemistry was tested for each property separately (**Table 12b**, marginal test) as well as by fitting all predictors into the most parsimonious model (**Table 12b**, sequential test). The best model for bacteria revealed the combination of soil moisture, K, Nmin, NH4, HWC, Mg, and pH as the best set of predictors (in decreasing order of importance) for explaining variations in community structure. For fungi, the model revealed K, soil moisture, Nmin, NO3, and P as the best set of predictors (**Table 12b**).

Table 12 Effects of tillage regime, crop residue management, and growing stage on soil physical and chemical soil properties.

Main test[a]	*P*	*Mg*	*Na*	*K*	*Ca*	*HWC*	*pH*	*moisture*	*NO3*	*NH4*	*Nmin*
	F(P)	F(P)	F(P)	F(P)	F(P)	F(P)	F(P)	F(P)	F(P)	F(P)	F(P)
Tillage	**8.1 (0.006°)**	1.3 (0.2)	0.01 (0.9)	**41.2 (0.00001°)**	**4.1 (0.05°)**	2.4 (0.1)	0.003 (0.9)	**31.9 (0.00001°)**	**5.2 (0.03°)**	1.3 (0.2)	**5.2 (0.03°)**
Residue	0.8 (0.4)	2.4 (0.1)	0.005 (0.9)	3.7 (0.06)	0.5 (0.5)	0.4 (0.5)	0.2 (0.6)	0.3 (0.6)	0.2 (0.7)	0.6 (0.5)	0.1 (0.7)
Stage	**2.4 (0.05°)**	**10.4 (0.00001°)**	**38.8 (0.00001°)**	**22.8 (0.00001°)**	**33.0 (0.00001°)**	**34.0 (0.00001°)**	0.7 (0.6)	**152.0 (0.00001°)**	**42.4 (0.00001°)**	**15.7 (0.00001°)**	**41 (0.00001°)**
Tillage*stage	**2.5 (0.05°)**	0.2 (0.9)	0.1 (1)	**4.5 (0.002°)**	0.2 (1)	0.9 (0.5)	1.4 (0.2)	**3.6 (0.01°)**	**4.0 (0.007°)**	0.3 (0.8)	**3.9 (0.007°)**
DistLM[b]	VC(P)	VC(P)	VC(P)	VC(P)	VC(P)	VC(P)	VC(P)	VC(P)	VC(P)	VC(P)	VC(P)
Bacteria (marginal test)	2.6 (0.00006°)	2.4 (0.0002°)	4.5 (0.00001°)	4.4 (0.00001°)	2.4 (0.0002°)	2.2 (0.0006°)	1.3 (0.07)	4.9 (0.00001°)	4.1 (0.00001°)	2.0 (0.002°)	4.1 (0.00001°)
Bacteria (sequential test)	1.02 (0.4)	**1.5 (0.01°)**	1.3 (0.06)	**4.9 (0.00001°)**	**1.6 (0.006°)**	**2.4 (0.0001°)**	**1.4 (0.03°)**	**4.9 (0.00001°)**	-	**2.1 (0.0003°)**	**2.1 (0.0003°)**
Fungi (marginal test)	3.0 (0.0005°)	3.6 (0.0001°)	5.3 (0.00001°)	5.7 (0.00001°)	2.6 (0.002°)	3.8 (0.00002°)	1.2 (0.2)	3.7 (0.00006°)	5.0 (0.00001°)	4.6 (0.00002°)	4.8 (0.00001°)
Fungi (sequential test)	**1.6 (0.03°)**	**2.5 (0.0003°)**	**4.3 (0.00001°)**	**5.7 (0.00001°)**	1.4 (0.06)	**2.6 (0.0003°)**	**1.4 (0.08°)**	**3.1 (0.00002°)**	**1.6 (0.03°)**	**4.9 (0.00001°)**	-

(a) Effects of main factors and their interactions as assessed by univariate permutational analysis of variance (PERMANOVA). Main factors represent tillage (CT, RT), residue management (R+, R-), and growing stage (1=seedling, 2=leaf development, 3=flowering, 4=tillering, 5=grain filling). *Vicia faba* correspond to stages 1, 2 and 3, and *Triticum aestivum* correspond to stages 4 and 5. Values represent the pseudo-F ratio (F) and the permutation-based level of significance (P). (b) Distance based-linear analysis (DISTLM) between microbial β-diversity and soil parameters. Values represent the variance component (VC) and the permutation-based level of significance (P). The significant results can be visualized in bold.

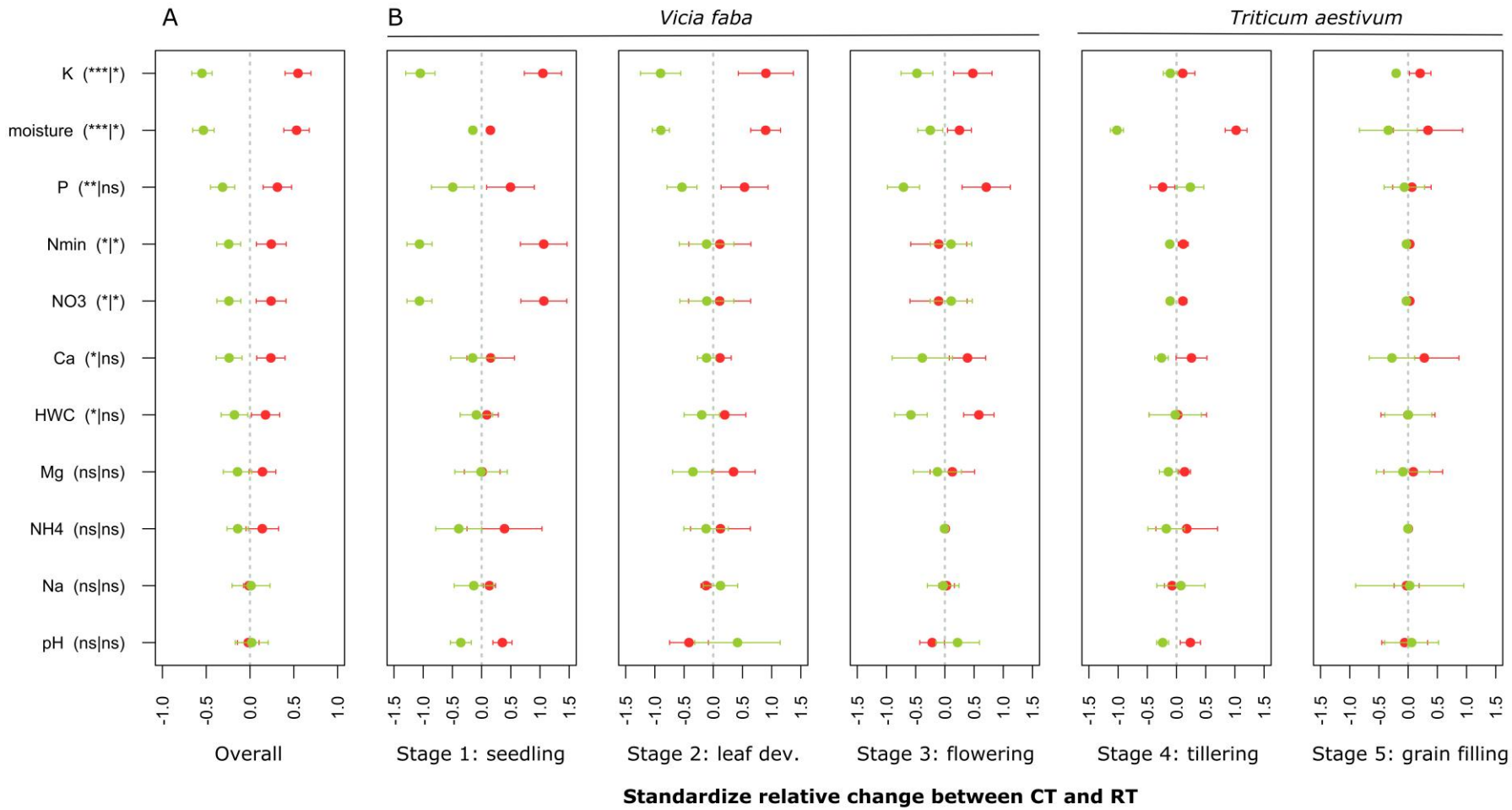


Figure 27 Standardized relative changes in physical and chemical soil properties combined over all growing stages (a) as well as for each individual stage (b) between CT (red) and RT (green). Data were z-transformed, representing values greater or smaller than the average across all samples. The significance of the PERMANOVA test is indicated in brackets: the first argument represents the significance of tillage effect and the second represents the significance of the interaction between tillage and growing stage. K=potassium, P=phosphorus, Nmin=mineral nitrogen, NO3=nitrate, Ca=calcium, HWC=hot water carbon, Mg=magnesium, NH4=ammonium, Na=sodium. CT, conventional tillage; RT, reduced tillage; *** q<0.001; ** q<0.01; * q<0.05; ns, not significant.

Individual response of taxa to tillage regime

The individual relative change in abundance of higher-order taxonomic groups (phylum and major classes of Proteobacteria, Ascomycota and Basidiomycota) to the tillage regime is shown in **Figure 28a**. Major groups of bacteria including Proteobacteria (α-, γ- and β-Proteobacteria), Bacteroidetes and Actinobacteria increased in relative abundance under CT, whereas Acidobacteria, Chloroflexi, Nitrospirae, Verrucomicrobia and δ-Proteobacteria increased in relative abundance under RT. In addition to these major groups, some bacterial candidate phyla including TM6 (recently called Dependentiae), Parcubacteria, Latescibacteria and Microgenomates increased in relative abundance under RT, whereas Saccharibacteria increased under CT. In the same way, major groups of fungi including Sordariomycetes, Dothideomycetes and Chytridiomycota increased in relative abundance under CT, whereas the relative abundance of Agaricomycetes, Basidiomycota, Pezizomycetes, Glomeromycota, Tremellomycetes and Leotiomycetes increased under RT. Tillage effects on higher-order taxonomic groups of bacteria and fungi showed a certain degree of variability over the growing stages, although none of the bacterial phyla or fungal classes revealed a statistically significant tillage × stage interaction term after correction for multiple testing (**Figure 28b**). Nevertheless, the majority of the bacterial phyla were not influenced by the tillage regime at the last growing stage investigated (stage 5). These results are in line with those shown in the **Table 10**.

The individual relative change in abundance to tillage regime was also determined at the OTU-level. In order to identify the statistically robust OTUs, only the OTUs occurring in at least 25% of the samples were included, leaving a total of 732 bacterial (43% of total bacterial OTUs) and 383 fungal (24% of total fungal OTUs) OTUs for analysis. Among those, 296 bacterial and 156 fungal OTUs showed a significant change in their relative abundance between CT and RT. A total of 199 and 97 bacterial OTUs responded positively to CT and RT, respectively, whereas 79 and 77 fungal OTUs responded positively to CT and RT, respectively. The distribution of these OTUs across the taxonomic hierarchy is shown in **Figure 29**. Several higher-order taxonomic groups such as Acidobacteria, Bacteroidetes, α- and β-Proteobacteria, and Glomeromycota showed a largely uniform response, i.e. most OTUs responding in the same direction to tillage, with a few exceptions. Other groups such as Verrucomicrobia, Chloroflexi, or Ascomycota showed a more heterogeneous response at the OTU level. On the basis of the existing scientific literature, the ecological relevance and potential lifestyles of the most salient tillage-sensitive taxa will be discussed in the next section.

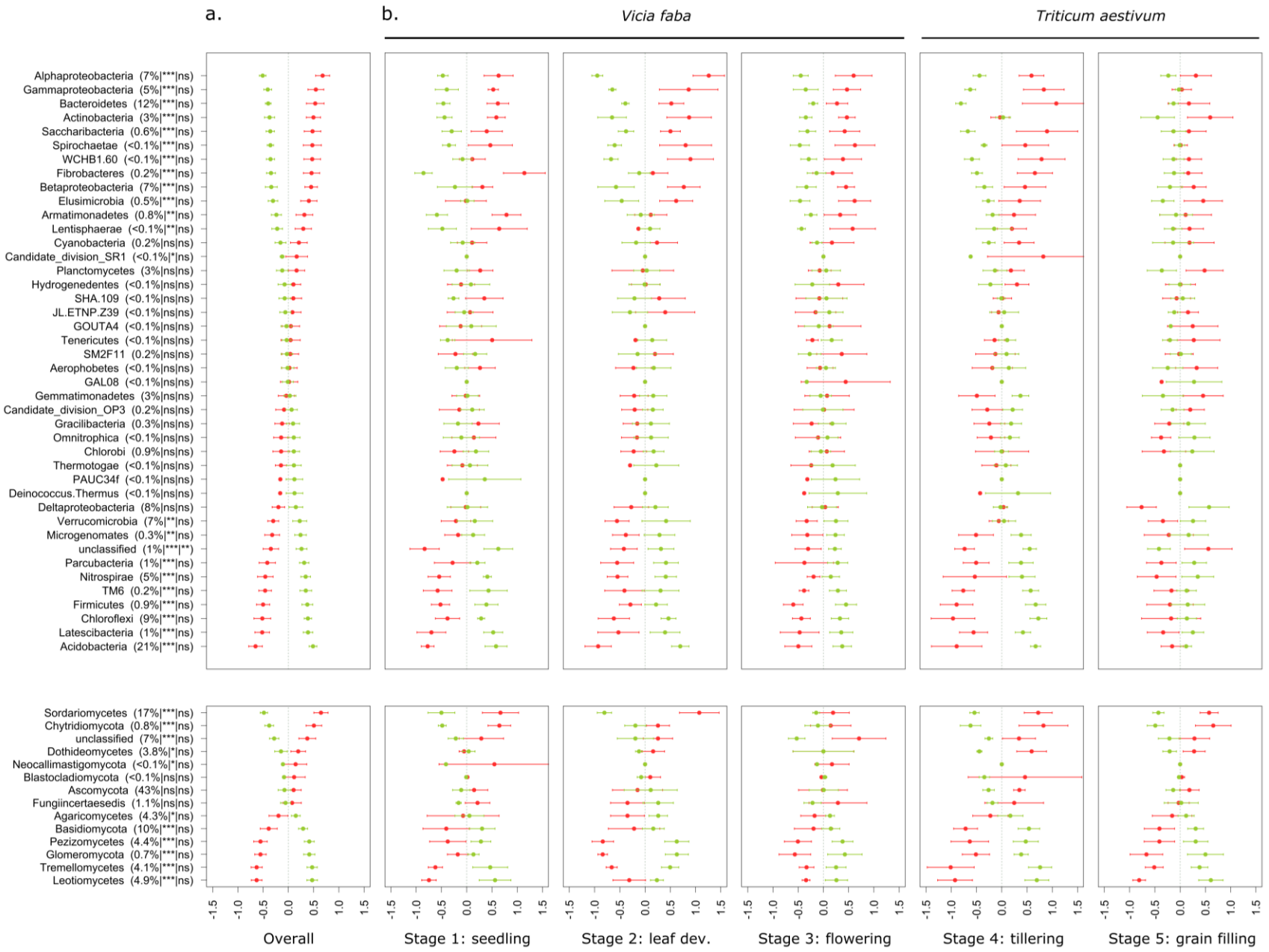


Figure 28 Standardized relative changes in abundance of higher-order taxonomic groups between CT (red) and RT (green) across all growing stages (a) and separately for each individual growing stage. Data were z-transformed, representing values greater or smaller than the average across all samples. The relative abundance as well as the significance of the PERMANOVA test is indicated in brackets: the first argument represents the relative abundance, the second is the significance of tillage effect and the third represents the significance of the interaction between tillage and growing stage. CT, conventional tillage; RT, reduced tillage; *** q<0.001; ** q<0.01; * q<0.05; ns, not significant.

6. Discussion

The effect of tillage on soil microbial α- and β-diversity

Overall, our findings evidenced CT more rich and less even than RT for both bacteria and fungi with no interaction between tillage and growing season (**Figure 26b & Table 11**). Based on the intermediate disturbance hypothesis (IDH) or "hump-back model" that describe the response of a community to stress (Giller et al., 1998), it could be assumed that under CT, plowing may act as an intermediate disturbance that is neither too rare nor too frequent, and result in an increased OTU diversity. Plowing mixes the different horizons and breaks down soil aggregates, which in turn releases organic matter and creates new ecological niches that allow colonization through minor or new species (Tilman, 1982). Indeed, the disturbance might favour r-strategic microorganisms, resulting in a more uneven community to be dominated be few OTUs with high competitive abilities under CT. Our estimation of diversity, however, was based on specific period of the growing season which was from March to June. Therefore, our results cannot be extrapolated to conclude that the community under CT is consistently more rich and less even over the whole year. To answer this question, further analyses are needed where the estimation diversity is based on period that covers the entire year. However, it is difficult to interpret shift in richness and evenness with respect to ecosystem functioning and crop productivity as relatively rare species can strongly influence certain soil processes. Consequently, we focus our discussion on the change in β-diversity and the taxonomic identity of tillage-sensitive taxa as they can play a beneficial or detrimental role in agroecosystem (Aislabie et al., 2013).

In agreement with the first hypothesis of our study, the tillage regime was a significant driver of microbial β-diversity (**Figure 26a, & Table 10**), which is consistent with the recent literature using high-resolution techniques (Carbonetto et al., 2014; Degrune et al., 2016; Dorr de Quadros et al., 2012; Jiménez-Bueno et al., 2016; Navarro-Noya et al., 2013; Sengupta and Dick, 2015). However, the direction of change of some microbial groups was not consistent with the other studies. For example, Carbonetto et al. (2014) reported higher relative abundance of Actinobacteria in no-tilled soil, whereas we evidenced higher abundance under CT when compared to RT (**Figure 28**). In the same study, the relative abundance of Nitrospirae was higher in tilled soil, whereas in our study the same phylum was higher under RT. Therefore, whereas there is a consensus that tillage alters soil microbial community structure, the response of individual groups appears to be very context-

specific and cannot be generalized across various agroecosystems. The response is largely dependent on the soil physical and chemical conditions induced by the tillage regime, which again differs among different soil types and under different climatic conditions. In our field study, six years after conversion from conventional to reduced tillage, soil nutrient and moisture contents were significantly lower under RT (**Figure 27**). Furthermore, previous investigations in the same field and at the same depth found that the soil's resistance to penetration as an estimation of soil density was more than twice as high under RT (40±6 kg cm-2) than under CT (15±1 kg cm-2).

Differences in β-diversity between CT and RT were mainly due to dissimilarity rather than dispersion, suggesting a similar level of stability between CT and RT. According to previous frameworks on environmental disturbances (Loreau et al., 2002; Rykiel, 1985), it could be hypothesized that the mechanical stress through plowing impacts the microbial community in a way that the heterogeneity in community structure across samples becomes higher under CT (less stability) than under RT. However, the time between the plowing and sampling may have been too long (around 3 months) for observing this likely immediate effect. In this context, the sampling schedule was not designed to measure initial resistance and long-term resilience of the microbial community to tillage, but rather to measure the impact of tillage at different growing stages of the crops. However, resistance and long-term resilience are important properties of such environmental disturbances and should be assessed in more detail in the future.

The effect of tillage over the growing season

According to the second hypothesis, we expected that tillage effects vary across the growing stage and that differences get smaller towards the end of the season. Indeed, the establishment of the root system over the season was expected to "dilute" the tillage regime effect on the microbial β-diversity. For both bacteria and fungi, a moderate effect of this interaction was noticed when compared to the tillage regime effect (**Table 10**). Moreover, for bacteria, the pairwise test revealed no tillage effect at the last stage of wheat. These results might suggest that bacteria and fungi differ in their response to tillage over the growing season. Fungi showed less resilience and a stronger crop effect, whereas bacteria appeared to be more resilient over the course of the season. The moderate interaction effect was further evidenced by looking at the individual responses of higher-order taxonomic groups, where a certain response variability was observed across the growing

stages, but no statistically significant interactions were identified (**Figure 28**). It could be argued, however, that corrections for multiple testing were potentially too conservative.

An interaction between tillage and growing stage would be expected as the establishment of the rooting system over time significantly influences the surrounding soil and may lead for examples to changes in the carbon source (root exudation), pH (ions release or uptake), water and oxygen contents (root water uptake and respiration), and nutrient availability (plant uptake and secretion of chelators to sequester micronutrients) (Philippot et al., 2013). In addition, the soil structure that determines pore connectivity and associated fluxes of oxygen and water, is also influenced by the root system (Bronick and Lal, 2005). Other factors may also contribute to the interaction effect between tillage and growing stage and include microbial resilience, i.e. the microbial community naturally recovers from the mechanical disturbance over time, as well as the climatic conditions that change over the season (e.g. temperature, moisture). The fact that only a moderate interaction effect was noticed might be linked to the sampling design. The samples were collected as close as possible to the stem, therefore the soil was not totally bulk soil, neither totally rhizosphere. We might assume that larger interaction effect would be detected if samples were collected within the rhizosphere, i.e. the narrow region of soil that is directly influenced by root secretions.

Our findings further evidenced substantial variability in physical and chemical soil parameters over the growing season between CT and RT (**Figure 27b**). The magnitude of tillage effect varied over time and differed with the studied parameters. This variability in the magnitude of changes between CT and RT over the growing season might be attributed to the establishment of root system that differ between CT and RT and that in turn might influence the water and nutrient flows through the soil profile. Previous measurement on the same experiment identified that under RT, the rooting system was limited at top soil mostly because of the presence of highly compacted soil layer below 10 cm, whereas under CT the rooting system was not limited and explored the whole soil profile (Eylenbosch et al., 2015). Consequently, over the growing season, the soil under CT at the studied depth was colonized by roots, whereas the soil under RT was not. In addition, the flows of water and nutrients were likely to be altered under RT due to the compaction, thus leading to different penetration dynamics under CT and RT over the growing season.

The impact of tillage over the growing season on the structure of microbial communities was previously investigated using lower resolution methods such as biochemical methods (Shi et al., 2013; Spedding et al., 2004; Zhang et al., 2012). Interactive effects between tillage and growing

season, however, were not consistent across these studies. Whereas Zhang et al. (2012) and Shi et al. (2013) reported that the tillage effect on the soil microbiota was dependent on the stage of growing season, Spedding et al. (2004) found no interaction effects between tillage and growing season. Again, the regional climatic conditions as well as the local edaphic properties including soil texture, structure, and moisture, may explain the discrepancy across different studies. For example, soil texture is one of the major determinants of how soil (and its inhabitants) responds to mechanical disturbance (Hartmann et al. 2014) and the water regime driven by climate were also found to strongly influence microbial diversity (Drenovsky et al., 2004; Ulrich and Becker, 2006). Therefore, it remains difficult to draw universally valid conclusions in that respect.

Potential interactions between microbial taxa and their environment

Tillage regime caused substantial changes in soil physical (moisture and aeration), chemical (nutrient availability and carbon accessibility) and biological (root system development) conditions. In our study, substantial differences in nutrient and moisture contents were recorded at deeper layer of soil (15-20 cm), with CT featuring higher nutrient and moisture contents than RT (**Figure 27a**). As mentioned earlier, the absence of plowing for the last 6 years under reduced tillage has led to the formation of highly compacted soil layer, resulting in alteration of the soil pore network that in turn might influence the nutrient and water flows through the soil profile as well as root penetration into the lower soil layers. Consequently, we expect that most of nutrients and moisture remained in the first centimeters of soil under RT, while under CT, the penetration of nutrients and moisture in deeper layer was facilitated by the higher occurrence of macrospores resulting from the alteration of the soil pore network by plowing (Lipiec et al., 2006). The quantity of crop residues was also likely to be different between CT and RT, resulting in higher availability of C source under CT when compared to RT where crop residues remained at top soil. Moreover, the quality of C was also likely to be different between CT and RT, with more recalcitrant material under CT (fresh crop residues added yearly by plowing).

All these changes in physical and chemical parameters between CT and RT were expected to induce substantial changes in the structure of microbial communities. Based on our third hypothesis, we speculate on the presence of some taxa in relation with soil physical and chemical conditions found under CT and RT. Here, we used taxonomy to infer on the presence of some taxa displaying specific lifestyles that can be related to environmental characteristics. Although such information on

lifestyles can be found at higher taxonomic levels (Philippot et al., 2010), there is an interest to go deeper in the taxonomy and identify members involved in more complex functions usually shown at lower taxonomic levels (Martiny et al., 2013). To date, however, describing the entire diversity of microbial communities with respect to the changes in environmental factors remains a challenge since we still have a limited understanding of the ecological attributes of many microbial taxa and many OTUs cannot be assigned at lower taxonomic levels. Consequently, we focused our analysis on the most salient examples.

According to the oligotrophy-copiotrophy framework previously outlined by Fierer et al. (2007), the higher nutrient status found under CT might explain the higher relative abundance of Proteobacteria (α, β, γ) and Bacteroidetes, bacterial groups that reportedly feature mainly copiotrophic lifestyles and thrive better under conditions of high nutrient availability. In contrast, the lower nutrient status found under RT might explain the higher relative abundance of Acidobacteria, which was reported to largely exhibit oligotrophic lifestyles and thrive better under conditions of lower nutrient availability (Fierer et al., 2007).

Several bacterial groups that are known to carry the ability to degrade recalcitrant C compounds such lignin found in crop residues, including α-, γ and β-Proteobacteria, as well as Actinobacteria (Goldfarb et al., 2011; Kameshwar and Qin, 2016), were significantly increased under CT. The putative ability to degrade complex organic matter under CT was also found at lower taxonomic resolution (**Figure 29**). Some members of genera *Flavobacterium* (**Figure 29**, clade #1) and *Cellvibrio* (clade #2), are known to be involved in lignocellulose degradation (Burgess, 2015; Jiménez et al., 2013; Koga et al., 1999), members of genus *Adhaeribacter* (clade #3) showed increase in soils receiving organic amendments, suggesting efficient usage of complex organic matter (Calleja-Cervantes et al., 2015), and members of *Actinoplanes* (clade #4) are found to be more abundant in leaf litter samples (Binh et al., 2011).

In RT soils, we identified an increased abundance of the phylum Nitrospirae (**Figure 28a**) and its genus *Nitrospira* (**Figure 29**, clade #5). Members of this group are nitrite-oxidizing bacteria (Daims et al., 2001; Juretschko et al., 1998) and exhibit largely oligotrophic characteristics (Nowka et al., 2015; Schramm et al., 1999). In addition, a recent study identified an increase of Nitrospirae in compacted soils (Hartmann et al., 2014) such that we can speculate that the increase relative abundance of Nitrospirae under RT indicated that these soils are less aerated than under CT as suggested by the strong difference in soil density mentioned above. Firmicutes were found to be

more abundant under RT, but most individual OTUs were not affected by tillage. We identified three OTUs responding positively to RT that were associated with endospore forming taxa such as *Paenibacillus* (aerobic) (clade #6) and *Clostridium* (anaerobic) (clade #7). Members of the Clostridiales (containing *Clostridium*) are metabolically diverse and may ferment sugars, starch, pectin, and cellulose under more oxygen-limited conditions (Goldfarb et al., 2011). Here again, we can speculate that an increased relative abundance of *Clostridium* could indicate more anaerobic microsites under RT.

Several groups of the recently suggested candidate phyla radiation (CPR) (Brown et al., 2015) differed in abundance between the tillage regimes. In general, members of the CPR have small streamlined genome, are versatile in their nutrient-spectrum (Wrighton et al., 2012), and exhibit potentially ectosymbiotic lifestyles, i.e. living on the surface of the host (Nelson and Stegen, 2015; Yeoh et al., 2015). These characteristics appear to favor adaptation to more nutrient poor, oligotrophic conditions, as they have even been found to be strongly enriched in highly oligotrophic environments such as permafrost (Frey et al. 2016) and deep sea sediments (Zhu et al., 2013). Therefore, we can speculate that the increased relative abundance of Parcubacteria (formerly OD1) and Microgenomates (formerly OP11) under RT (**Figure 29a**) is another indication that these soils are likely more nutrient-limited than under CT.

Fungi, known as major drivers of organic matter decomposition, showed substantial variability in community structure between CT and RT and they also showed less resilience towards the end of the growing season (**Table 10**). Although fungi are usually sensitive to mechanical disturbance that cause damages to their hyphal network, some major groups such as Chytridiomycota and Sordariomycetes (major class of Ascomycota) depicted higher abundance under CT (**Figure 28a**).

Members of Chytridiomycota are commonly found in soil and exhibit either saprobic or parasitic lifestyles, but the ecological relevance of Chytridiomycota in agroecosystems is still poorly understood. Most of them are unicellular and only few show multicellular hyphal growth, which could be one reason why they are relatively more abundant under CT as they are less susceptible to mechanical disturbance. A recent study has emphasized their potential ability to degrade cellulose, a major component of plant cell wall, suggesting an important role in C-decomposition (Kameshwar and Qin, 2016).

Basidiomycota, a vast and complex group of fungi containing a large number of saprophytic (wood decayers, litter decomposer), ectomycorrhizal, and parasitic fungi (Watkinson, 2008), was found to

be higher under RT (**Figure 28a**). Most of the abundant members of this phylum responded positively to RT (**Figure 29**). Typically, saprophytic members were recognized to degrade complex components such as lignin contained in plant litter and wood more rapidly than other fungi (Osono and Takeda, 2002). The two major classes of Basidiomycota belong to Agaricomycetes (**Figure 29**, clade #8) and Tremellomycetes (**Figure 29**, clade #9), and responded positively to RT (**Figure 28a**). Notably, Agaricomycetes are critical decomposers and contain the 'soft', 'brown' and 'white' rot fungi that produce hydrogen peroxide and enzymes to degrade complex plant compounds including cellulose and lignin (Kameshwar and Qin, 2016). At finer taxonomic resolution we identified three major fungi including *Guehomyces_pullulans* (clade #10), and two species of *Cryptococcus* (C. terricola and C. aerius) (clades #11 and #12). These organisms are single-celled microorganisms (yeast) and known to feature a wide range of enzymatic activities (Martinez et al., 2016). Yeast have developed adaptation strategies to overcome notably low-nutrient and oxygen-poor conditions (Fonseca and Inácio, 2006), for instance those found in oligotrophic lake in Patagonia (Brandão et al., 2011) and glacial areas (Buzzini et al., 2012, Frey et al., 2016). Again, the presence of such oligotrophic organisms might be related to the more nutrient- and oxygen-limited conditions found under RT when compared to CT.

The Glomeromycota, a fungal group of significant ecological and economic importance, was found to be more abundant under RT. Members of this group contain arbuscular mycorrhizal fungi (or AMF) that form symbiotic associations with the majority of vascular plants and significantly increase nutrient availability for the host plant, and, thus, play a crucial role in agroecosystem functioning (Douds and Millner, 1999). It has previously been shown that this group of fungi favored under reduced tillage (Säle et al., 2015).

Ascomycota is the largest fungal phylum and display a large and wide range of life-history strategy. Although no overall tillage effect on this group was noticed **Figure 28a**), the individual OTUs belonging to Pezizales responded uniformly and positively to RT (**Figure 29**, clade #13), whereas the response of individual OTUs within Sordariomycetes were less uniform (clade #14). Sordariomycetes (clade #14) is one of the largest classes of Ascomycota and feature a wide range of lifestyles such as pathogens and endophytes of plants, and mycoparasites (Zhang et al., 2006). Although this group responded positively to CT (**Figure 28a**), the individual response to tillage at the OTU level differed substantially (**Figure 29**, clade #14). Among the most abundant OTUs, *Podospora* and *Schizothecium* genus were identified to be more abundant under CT (**Figure 29**, clade #15 and

#16). Both of them, phylogenetically similar (Cai et al., 2005), belong to coprophilous, a type of saprobic fungi that grow on animal dung. We further identified *Fusarium graminearum* (**Figure 29**, clade #17), the causative agent of Fusarium head blight of wheat (Bottalico and Perrone, 2002), to be more abundant under CT. As reported by Booth (1971), *Fusarium graminearum* can survive saprophytically on a wide range of gramineous host debris, such as wheat residues. As our samples were taken at a depth between 15 and 20 cm, the higher relative abundance of *Fusarium graminearum* observed under CT might be due to the presence of crop residues from previous wheat crops at this depth, while crop residues remain in the topsoil (<10 cm) under RT.

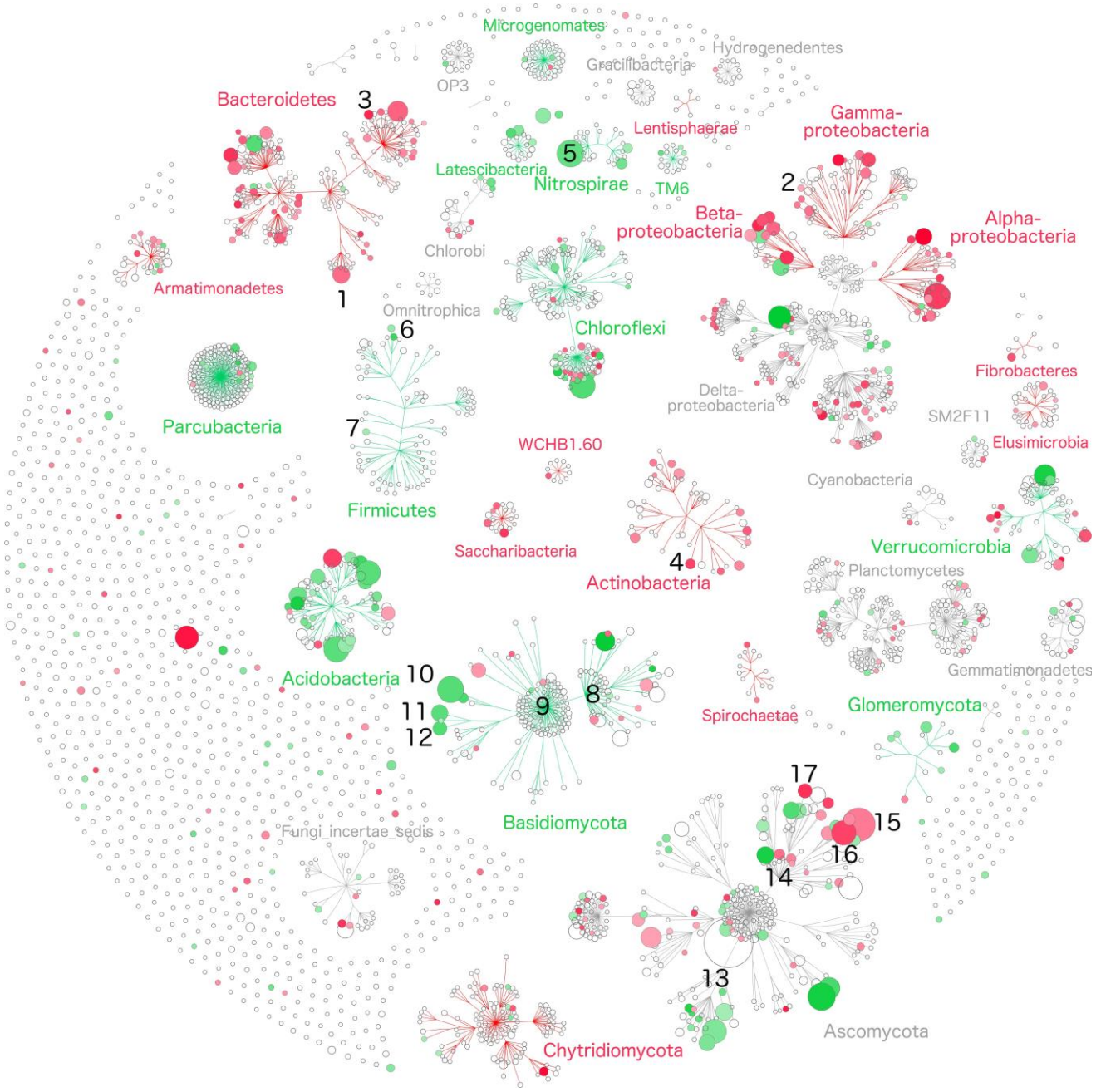


Figure 29 Taxonomic networks showing the distribution of bacterial and fungal OTU across the taxonomic hierarchy. Nodes correspond to OTUs and nodes size corresponds to their relative abundance (square root) in the dataset. Edges (lines connecting the nodes) represent the taxonomic path from phylum to OTU level, whereas OTUs are placed at the level of the lowest possible assignment. The response of individual OTUs to tillage was mapped onto the taxonomic network with green nodes corresponding to OTUs responding positively to reduced tillage (RT) and red nodes corresponding to OTUs responding positively to conventional tillage (CT). The color intensity was related to the strength of the tillage effect and only significant nodes were color-coded ($q<0.05$). All OTUs are visualized, but only the robust OTUs occurring in at least 25% of all samples (= 20 samples) were statistically evaluated.

7. Conclusion

Here, we explored the response of the soil microbial community structure to two contrasting tillage regimes over different growing stages of two crops using high-resolution metabarcoding techniques. Our study emphasized potential consequences of compaction under reduced tillage, leading to shifts in soil physical and chemical properties when compared to conventional tillage, which in turn influence diversity and structure of the microbiome. More specifically, we reported lower nutrient and moisture contents under reduced tillage, promoting microbial taxa featuring copiotrophic lifestyles under conventional tillage and taxa featuring oligotrophic lifestyles under reduced tillage. The higher quantity of fresh crop residues under conventional tillage seemed to favor the presence of certain taxa that feature specific ability to degrade recalcitrant material. We further reported a moderate influence of the growing season on the tillage regime effect. The response of bacterial communities to tillage regime according to the growing season differed from the response of fungal communities. The structure of bacterial communities at the end of the cropping season was less influenced by tillage regime than fungi. Although changes in α- and β-diversity were noticed, our study provided no evidences on the impact of diversity changes on the functioning of the agroecosystem. Further studies are needed to relate the microbial diversity changes with the functioning of agroecosystem through the assessment of diverse ecosystem functions.

Acknowledgments

We thank the University of Liège Gembloux Agro-Bio Tech, and particularly the TERRA-AgricultureIsLife research platform, for funding this research project. We greatly acknowledged the field teams (farmers, technicians and researchers) of the experimental farm for the continuous high-quality management of the field experiment. We also thank the laboratory team of Christian Roisin (CRAw) for the determination of NO3 and NH4+ in soil samples and the measurement of soil resistance to penetration. We acknowledge the Genetic Diversity Center (GDC) at ETH Zurich and the CAMI at Gembloux Agro-Bio Tech for providing access to high-performance computing facilities.

8. References

Aislabie, J., Deslippe, J.R., Dymond, J., others, 2013. Soil microbes and their contribution to soil services. Ecosyst. Serv. N. Z. Cond. Trends 143161.

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. Agric. Ecosyst. Environ. 74, 19–31.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Bending, G.D., Turner, M.K., Jones, J.E., 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. Soil Biol. Biochem. 34, 1073–1082.

Berg, G., 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biotechnol. 84, 11–18. doi:10.1007/s00253-009-2092-7

Binh, C.T.T., Otoguro, M., Miyadoh, S., Hang, D.T., Ando, K., Sakiyama, Y., Luong, D.T., Van Hop, D., 2011. Taxonomic and ecological studies of actinomycetes from Vietnam: isolation and genus-level diversity.

Bottalico, A., Perrone, G., 2002. Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur. J. Plant Pathol. 108, 611–624.

Brandão, L.R., Libkind, D., Vaz, A.B.M., Espírito Santo, L.C., Moliné, M., de García, V., van Broock, M., Rosa, C.A., 2011. Yeasts from an oligotrophic lake in Patagonia (Argentina): diversity, distribution and synthesis of photoprotective compounds and extracellular enzymes: Yeast diversity of Nahuel Huapi Lake. FEMS Microbiol. Ecol. 76, 1–13. doi:10.1111/j.1574-6941.2010.01030.x

Brockett, B.F., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biol. Biochem. 44, 9–20.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3–22.

Brown, C.T., Hug, L.A., Thomas, B.C., Sharon, I., Castelle, C.J., Singh, A., Wilkins, M.J., Wrighton, K.C., Williams, K.H., Banfield, J.F., 2015. Unusual biology across a group comprising more than 15% of domain Bacteria. Nature 523, 208–211. doi:10.1038/nature14486

Burgess, R.A., 2015. Synergistic degradation of lignocellulose by fungi and bacteria in boreal forest soil (Thesis).

Buzzini, P., Branda, E., Goretti, M., Turchetti, B., 2012. Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. FEMS Microbiol. Ecol. 82, 217–241. doi:10.1111/j.1574-6941.2012.01348.x

Cai, L., Jeewon, R., Hyde, K.D., 2005. Phylogenetic evaluation and taxonomic revision of Schizothecium based on ribosomal DNA and protein coding genes. Fungal Divers.

Calleja-Cervantes, M.E., Menéndez, S., Fernández-González, A.J., Irigoyen, I., Cibriáin-Sabalza, J.F., Toro, N., Aparicio-Tejo, P.M., Fernández-López, M., 2015. Changes in soil nutrient content and bacterial community after 12 years of organic amendment application to a vineyard. Eur. J. Soil Sci. 66, 802–812. doi:10.1111/ejss.12261

Carbonetto, B., Rascovan, N., Álvarez, R., Mentaberry, A., Vázquez, M.P., 2014. Structure, Composition and Metagenomic Profile of Soil Microbiomes Associated to Agricultural Land Use and Tillage Systems in Argentine Pampas. PLoS ONE 9, e99949. doi:10.1371/journal.pone.0099949

Chau, J.F., Bagtzoglou, A.C., Willig, M.R., 2011. The effect of soil texture on richness and diversity of bacterial communities. Environ. Forensics 12, 333–341.

Clarke, K., Gorley, R., 2006. Primer v6: User Manual/Tutorial. PRIMER-E. Plymouth, UK.

Daims, H., Nielsen, J.L., Nielsen, P.H., Schleifer, K.-H., Wagner, M., 2001. In Situ Characterization ofNitrospira-Like Nitrite-Oxidizing Bacteria Active in Wastewater Treatment Plants. Appl. Environ. Microbiol. 67, 5273–5284.

Degrune, F., Theodorakopoulos, N., Dufrêne, M., Colinet, G., Bodson, B., Hiel, M.-P., Taminiau, B., Nezer, C., Daube, G., Vandenbol, M., 2016. No favorable effect of reduced tillage on microbial community diversity in a silty loam soil (Belgium). Agric. Ecosyst. Environ. 224, 12–21.

Dorr de Quadros, P., Zhalnina, K., Davis-Richardson, A., Fagen, J.R., Drew, J., Bayer, C., Camargo, F.A., Triplett, E.W., 2012. The effect of tillage system and crop rotation on soil microbial diversity and composition in a subtropical Acrisol. Diversity 4, 375–395. doi:10.3390/d4040375

Douds, D.D., Millner, P.D., 1999. Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. Agric. Ecosyst. Environ. 74, 77–93.

Drenovsky, R.E., Vo, D., Graham, K.J., Scow, K.M., 2004. Soil Water Content and Organic Carbon Availability Are Major Determinants of Soil Microbial Community Composition. Microb. Ecol. 48, 424–430. doi:10.1007/s00248-003-1063-2

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.

Eylenbosch, D., Fernández Pierna, J.A., Baeten, V., Bodson, B., 2015. Use of NIR hyperspectral imaging and chemometrics to quantify roots and crop residues in soil.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.

Fonseca, A., Inácio, J., 2006. Phylloplane yeasts, in: Biodiversity and Ecophysiology of Yeasts. Springer, pp. 263–301.

Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F., Hartmann, M., 2016. Microbial diversity in European alpine permafrost and active layers. FEMS Microbiol. Ecol. 92, fiw018. doi:10.1093/femsec/fiw018

Frey, S.D., Drijber, R., Smith, H., Melillo, J., 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biol. Biochem. 40, 2904–2907.

Fruchterman, T.M., Reingold, E.M., 1991. Graph drawing by force-directed placement. Softw. Pract. Exp. 21, 1129–1164.

Ghani, A., Dexter, M., Perrott, K.W., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol. Biochem. 35, 1231–1243.

Giller, K.E., Witter, E., Mcgrath, S.P., 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem. 30, 1389–1414.

Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil Type Is the Primary Determinant of the Composition of the Total and Active Bacterial Communities in Arable Soils. Appl. Environ. Microbiol. 69, 1800–1809. doi:10.1128/AEM.69.3.1800-1809.2003

Goldfarb, K.C., Karaoz, U., Hanson, C.A., Santee, C.A., Bradford, M.A., Treseder, K.K., Wallenstein, M.D., Brodie, E.L., 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Front. Microbiol. 2, 94.

Gower, J.C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53, 325–338.

Hao, X., Jiang, R., Chen, T., 2011. Clustering 16S rRNA for OTU prediction: a method of unsupervised Bayesian clustering. Bioinformatics 27, 611–618.

Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J. 9, 1177–1194. doi:10.1038/ismej.2014.210

Hartmann, M., Howes, C.G., Abarenkov, K., Mohn, W.W., Nilsson, R.H., 2010. V-Xtractor: an open-source, high-throughput software tool to identify and extract hypervariable regions of small subunit (16S/18S) ribosomal RNA gene sequences. J. Microbiol. Methods 83, 250–3. doi:10.1016/j.mimet.2010.08.008

Hartmann, M., Niklaus, P.A., Zimmermann, S., Schmutz, S., Kremer, J., Abarenkov, K., Lüscher, P., Widmer, F., Frey, B., 2014. Resistance and resilience of the forest soil microbiome to logging-associated compaction. ISME J. 8, 226–244.

Héry, M., Singer, A.C., Kumaresan, D., Bodrossy, L., Stralis-Pavese, N., Prosser, J.I., Thompson, I.P., Murrell, J.C., 2007. Effect of earthworms on the community structure of active methanotrophic bacteria in a landfill cover soil. ISME J. 2, 92–104. doi:10.1038/ismej.2007.66

Hobbs, P.R., Sayre, K., Gupta, R., 2008. The role of conservation agriculture in sustainable agriculture. Philos. Trans. R. Soc. B Biol. Sci. 363, 543–555. doi:10.1098/rstb.2007.2169

Houlden, A., Timms-Wilson, T.M., Day, M.J., Bailey, M.J., 2008. Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. FEMS Microbiol. Ecol. 65, 193–201. doi:10.1111/j.1574-6941.2008.00535.x

Jiménez, D.J., Korenblum, E., Elsas, J.D. van, 2013. Novel multispecies microbial consortia involved in lignocellulose and 5-hydroxymethylfurfural bioconversion. Appl. Microbiol. Biotechnol. 98, 2789–2803. doi:10.1007/s00253-013-5253-7

Jiménez-Bueno, N.G., Valenzuela-Encinas, C., Marsch, R., Ortiz-Gutiérrez, D., Verhulst, N., Govaerts, B., Dendooven, L., Navarro-Noya, Y.E., 2016. Bacterial indicator taxa in soils under different long-term agricultural management. J. Appl. Microbiol. n/a-n/a. doi:10.1111/jam.13072

Juretschko, S., Timmermann, G., Schmid, M., Schleifer, K.-H., Pommerening-Röser, A., Koops, H.-P., Wagner, M., 1998. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: Nitrosococcus mobilis and Nitrospira-like bacteria as dominant populations. Appl. Environ. Microbiol. 64, 3042–3051.

Käll, L., Storey, J.D., Noble, W.S., 2009. QVALITY: non-parametric estimation of q-values and posterior error probabilities. Bioinformatics 25, 964–966.

Kameshwar, A. kumar S., Qin, W., 2016. Recent Developments in Using Advanced Sequencing Technologies for the Genomic Studies of Lignin and Cellulose Degrading Microorganisms. Int. J. Biol. Sci. 12, 156–171. doi:10.7150/ijbs.13537

Kibblewhite, M.G., Ritz, K., Swift, M.J., 2008. Soil health in agricultural systems. Philos. Trans. R. Soc. B Biol. Sci. 363, 685–701.

Koga, S., Ogawa, J., Choi, Y.-M., Shimizu, S., 1999. Novel bacterial peroxidase without catalase activity from Flavobacterium meningosepticum: purification and characterization. Biochim. Biophys. Acta BBA-Protein Struct. Mol. Enzymol. 1435, 117–126.

Kowalchuk, G.A., Buma, D.S., de Boer, W., Klinkhamer, P.G., van Veen, J.A., 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie Van Leeuwenhoek 81, 509–520.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. Appl. Environ. Microbiol. 75, 5111–5120. doi:10.1128/AEM.00335-09

Lauber, C.L., Ramirez, K.S., Aanderud, Z., Lennon, J., Fierer, N., 2013. Temporal variability in soil microbial communities across land-use types. ISME J. 7, 1641–1650. doi:10.1038/ismej.2013.50

Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., Pierre, K.L., Risch, A.C., Seabloom, E.W., Schütz, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proc. Natl. Acad. Sci. 112, 10967–10972. doi:10.1073/pnas.1508382112

Lipiec, J., Kuś, J., Słowińska-Jurkiewicz, A., Nosalewicz, A., 2006. Soil porosity and water infiltration as influenced by tillage methods. Soil Tillage Res. 89, 210–220. doi:10.1016/j.still.2005.07.012

Loreau, M., Downing, A., Emmerson, M., Gonzalez, A., Hughes, J., Inchausti, P., Joshi, J., Norberg, J., Sala, O., 2002. A new look at the relationship between diversity and stability. Biodivers. Ecosyst. Funct. Synth. Perspect. Oxf. Univ. Press Oxf. 79–91.

Lüdemann, H., Arth, I., Liesack, W., 2000. Spatial Changes in the Bacterial Community Structure along a Vertical Oxygen Gradient in Flooded Paddy Soil Cores. Appl. Environ. Microbiol. 66, 754–762. doi:10.1128/AEM.66.2.754-762.2000

Maidak, B.L., Olsen, G.J., Larsen, N., Overbeek, R., McCaughey, M.J., Woese, C.R., 1996. The Ribosomal Database Project (RDP). Nucleic Acids Res. 24, 82–85. doi:10.1093/nar/24.1.82

Martinez, A., Cavello, I., Garmendia, G., Rufo, C., Cavalitto, S., Vero, S., 2016. Yeasts from sub-Antarctic region: biodiversity, enzymatic activities and their potential as oleaginous microorganisms. Extremophiles 20, 759–769. doi:10.1007/s00792-016-0865-3

Martiny, A.C., Treseder, K., Pusch, G., 2013. Phylogenetic conservatism of functional traits in microorganisms. ISME J. 7, 830–838. doi:10.1038/ismej.2012.160

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82, 290–297.

Montgomery, D.R., 2007. Soil erosion and agricultural sustainability. Proc. Natl. Acad. Sci. 104, 13268–13272.

Murphy, R.P., Montes-Molina, J.A., Govaerts, B., Six, J., van Kessel, C., Fonte, S.J., 2016. Crop residue retention enhances soil properties and nitrogen cycling in smallholder maize systems of Chiapas, Mexico. Appl. Soil Ecol. 103, 110–116.

Nannipieri, P., Badalucco, L., 2003. Biological processes, in: Handbook of Processes and Modelling in the Soil-Plant System. Benbi, D.K., Nieder, R., Binghamton, NY, pp. 57–82.

Navarro-Noya, Y.E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M.C., Valenzuela-Encinas, C., Jiménez-Bueno, N., Verhulst, N., Govaerts, B., Dendooven, L., 2013. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biol. Biochem. 65, 86–95. doi:10.1016/j.soilbio.2013.05.009

Nelson, W.C., Stegen, J.C., 2015. The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle. Front. Microbiol. 6. doi:10.3389/fmicb.2015.00713

Nilsson, R.H., Veldre, V., Hartmann, M., Unterseher, M., Amend, A., Bergsten, J., Kristiansson, E., Ryberg, M., Jumpponen, A., Abarenkov, K., 2010. An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. Fungal Ecol. 3, 284–287.

Nowka, B., Daims, H., Spieck, E., 2015. Comparison of oxidation kinetics of nitrite-oxidizing bacteria: nitrite availability as a key factor in niche differentiation. Appl. Environ. Microbiol. 81, 745–753.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J., Suggests, M., 2007. The vegan package. Community Ecol. Package 631–637.

Osono, T., Takeda, H., 2002. Comparison of Litter Decomposing Ability among Diverse Fungi in a Cool Temperate Deciduous Forest in Japan. Mycologia 94, 421–427. doi:10.2307/3761776

Panettieri, M., Knicker, H., Murillo, J.M., Madejón, E., Hatcher, P.G., 2014. Soil organic matter degradation in an agricultural chronosequence under different tillage regimes evaluated by organic matter pools, enzymatic activities and CPMAS ^{1}^{3}C NMR. Soil Biol. Biochem. 78, 170–181.

Philippot, L., Andersson, S.G., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., Hallin, S., 2010. The ecological coherence of high bacterial taxonomic ranks. Nat. Rev. Microbiol. 8, 523–529.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., 2013. Going back to the roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789–799. doi:10.1038/nrmicro3109

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. doi:10.1093/nar/gks1219

Quince, C., Lanzén, A., Curtis, T., Davenport, R., Hall, N., Head, I., Read, L., Sloan, W., 2009. Accurate determination of microbial diversity from 454 pyrosequencing data. Nat. Methods 6, 639–641. doi:10.1038/nmeth.1361

R Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rykiel, E.J., 1985. Towards a definition of ecological disturbance. Aust. J. Ecol. 10, 361–365.

Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., van der Heijden, M.G., Oehl, F., 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biol. Biochem. 84, 38–52.

Salinas-Garcia, J.R., Baez-Gonzalez, A.D., Tiscareno-Lopez, M., Rosales-Robles, E., 2001. Residue removal and tillage interaction effects on soil properties under rain-fed corn production in Central Mexico. Soil Tillage Res. 59, 67–79.

Schloss, P.D., 2009. A High-Throughput DNA Sequence Aligner for Microbial Ecology Studies. PLoS ONE 4, e8230. doi:10.1371/journal.pone.0008230

Schramm, A., de Beer, D., van den Heuvel, J.C., Ottengraf, S., Amann, R., 1999. Microscale Distribution of Populations and Activities ofNitrosospira and Nitrospira spp. along a Macroscale Gradient in a

Nitrifying Bioreactor: Quantification by In Situ Hybridization and the Use of Microsensors. Appl. Environ. Microbiol. 65, 3690–3696.

Scopel, E., Triomphe, B., Affholder, F., Silva, F.A.M.D., Corbeels, M., Xavier, J.H.V., Lahmar, R., Recous, S., Bernoux, M., Blanchart, E., Mendes, I. de C., Tourdonnet, S.D., 2012. Conservation agriculture cropping systems in temperate and tropical conditions, performances and impacts. A review. Agron. Sustain. Dev. 33, 113–130. doi:10.1007/s13593-012-0106-9

Sengupta, A., Dick, W.A., 2015. Bacterial Community Diversity in Soil Under two Tillage Practices as Determined by Pyrosequencing. Microb. Ecol. 70, 853–859. doi:10.1007/s00248-015-0609-4

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res. 13, 2498–2504. doi:10.1101/gr.1239303

Shi, Y., Lalande, R., Hamel, C., Ziadi, N., Gagnon, B., Hu, Z., 2013. Seasonal variation of microbial biomass, activity, and community structure in soil under different tillage and phosphorus management practices. Biol. Fertil. Soils 49, 803–818. doi:10.1007/s00374-013-0773-y

Siddiqui, Z.A., Akhtar, M.S., Futai, K. (Eds.), 2008. Mycorrhizae: sustainable agriculture and forestry. Springer, Dordrecht.

Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Sci. Soc. Am. J. 63, 1350–1358.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. Soil Sci. Soc. Am. J. 70, 555. doi:10.2136/sssaj2004.0347

Smith, B., Wilson, J.B., 1996. A consumer's guide to evenness indices. Oikos 70–82.

Spedding, T.A., Hamel, C., Mehuys, G.R., Madramootoo, C.A., 2004. Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. Soil Biol. Biochem. 36, 499–512.

Storey, J.D., 2002. A direct approach to false discovery rates. J. R. Stat. Soc. Ser. B Stat. Methodol. 64, 479–498.

Tilman, D., 1982. Resource Competition and Community Structure. Princeton University Press Princeton, NJ.

Ulrich, A., Becker, R., 2006. Soil parent material is a key determinant of the bacterial community structure in arable soils. FEMS Microbiol. Ecol. 56, 430–443.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Appl. Environ. Microbiol. 73, 5261–5267. doi:10.1128/AEM.00062-07

Watkinson, S.C., 2008. Basidiomycota, in: John Wiley & Sons, Ltd (Ed.), Encyclopedia of Life Sciences. John Wiley & Sons, Ltd, Chichester, UK.

Wrighton, K.C., Thomas, B.C., Sharon, I., Miller, C.S., Castelle, C.J., VerBerkmoes, N.C., Wilkins, M.J., Hettich, R.L., Lipton, M.S., Williams, K.H., others, 2012. Fermentation, hydrogen, and sulfur metabolism in multiple uncultivated bacterial phyla. Science 337, 1661–1665.

Yeoh, Y.K., Sekiguchi, Y., Parks, D.H., Hugenholtz, P., 2015. Comparative Genomics of Candidate Phylum TM6 Suggests That Parasitism Is Widespread and Ancestral in This Lineage. Mol. Biol. Evol. msv281. doi:10.1093/molbev/msv281

Zhang, B., He, H., Ding, X., Zhang, X., Zhang, X., Yang, X., Filley, T.R., 2012. Soil microbial community dynamics over a maize (Zea mays L.) growing season under conventional-and no-tillage practices in a rainfed agroecosystem. Soil Tillage Res. 124, 153–160.

Zhang, N., Castlebury, L.A., Miller, A.N., Huhndorf, S.M., Schoch, C.L., Seifert, K.A., Rossman, A.Y., Rogers, J.D., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Sung, G.-H., 2006. An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. Mycologia 98, 1076–1087. doi:10.3852/mycologia.98.6.1076

Zhu, D., Tanabe, S.-H., Yang, C., Zhang, W., Sun, J., 2013. Bacterial Community Composition of South China Sea Sediments through Pyrosequencing-Based Analysis of 16S rRNA Genes. PLOS ONE 8, e78501. doi:10.1371/journal.pone.0078501

Chapter VI. General discussion, conclusions, and prospects

1. General discussion

Back to the Walloon context

Conservation tillage in Europe: a tricky affair

Before diving into the heart of the matter and discussing our results regarding the responses of microbial diversity and community structure to different tillage regimes and crop residue management practices, let us return to the European context, as well as the local pedological and climate contexts of Wallonia.

In Europe, the beneficial effect of conservation tillage practices on soil quality is still under debate (Soane et al., 2012). Notably, a recent study (Basch et al., 2015) has evidenced the importance of interactions between crops, soil type, and regional climate conditions in determining the success of conservation tillage in Europe. The authors of this study have reviewed both the benefits and disadvantages of such practices that may occur in some situations (**Figure 30**).

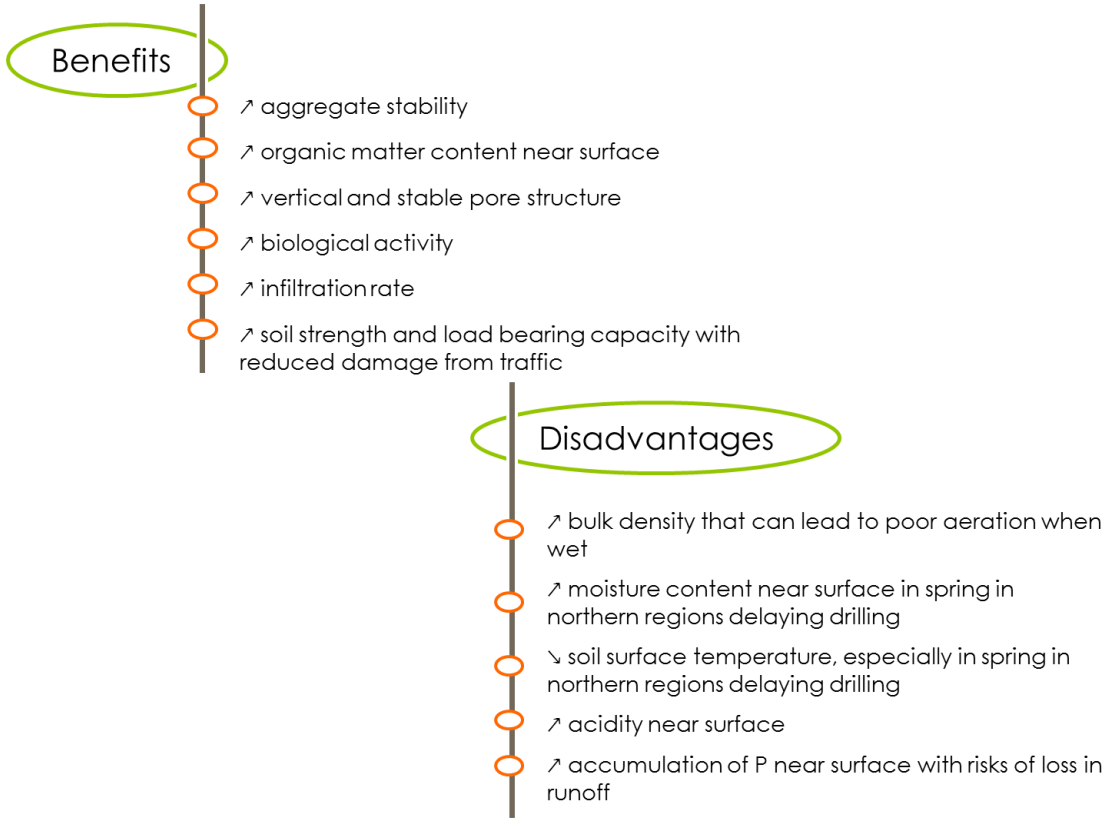


Figure 30 Summary of the most frequently reported changes in soil properties after several years of no-till (according to Basch et al., (2015)).

In addition, the crop productivity is a key factor that will determine the implementation of conservation tillage practices by farmers. Even if economic and environmental advantages motivate the use of soil conservation tillage, crop productivity has to be maintained at a higher or at least similar level than with conventional tillage. A recent meta-analysis showed that, on average in Europe, the adoption of conservation tillage practices tends to decrease crop yields (Labreuche et al., 2014). However, this decrease is larger when using strict no-till, while with intermediate conservation tillage practices, such as reduced tillage, the yield reduction is observed only for soil structure-sensitive crops, for instance maize and sugar beet. As a result, the option of implementing conservation tillage practices is not obvious and requires knowledge, technicity and expertise on the part of farmers.

In Wallonia specifically, several decades of agronomic research have produced evidence that in certain cases reduced tillage can detrimentally affect the soil structure and cause soil compaction[3]. This can adversely affect the productivity of certain profitable crops (e.g. sugar beet and potato) that are highly sensitive to soil structure. **Figure 31** (according to Roisin, 1997) clearly shows that in Wallonia, the effect of RT on sugar beet productivity has been quite variable.

The soil compaction, mostly caused by farm animals and heavy machines is considered as a serious problem in intensive agriculture worldwide (Hamza and Anderson, 2005). It occurs under certain climatic and edaphic conditions and may prevent the success of conservation tillage.

Among the wide range of factors influencing soil compaction processes, the **soil water content** (mostly drove by the climate regime) is the most important (Soane and van Ouwerkerk, 2013). The sensitivity of soil to compaction is increased with increasing soil moisture content. As a result, cultivation operations have to be schedule at the appropriate moisture content.

In Belgium, however, soil is regularly humid because of the oceanic temperate climate (humid summer and rainy winter), and schedule cultivation operations at the appropriate moisture content is technically difficult. As a result, we assume that under RT, the bulk density of soil layer below 10 cm increases over time with traffic (even if less frequent than under CT) and inappropriate operations timing, whereas, under CT, the soil layer between 0 and 25 cm is frequently disturbed and no soil compaction occurs within this layer. Under CT, however, the traffic can cause soil

[3] It refers to the processes by which the soil grains are rearranged to decrease porosity and permeability, thereby increasing the soil strength.

compaction in deeper soil and lead to the formation of the “plow pan”, which in turn prevent the water infiltration and the plant root development in deeper soil layers.

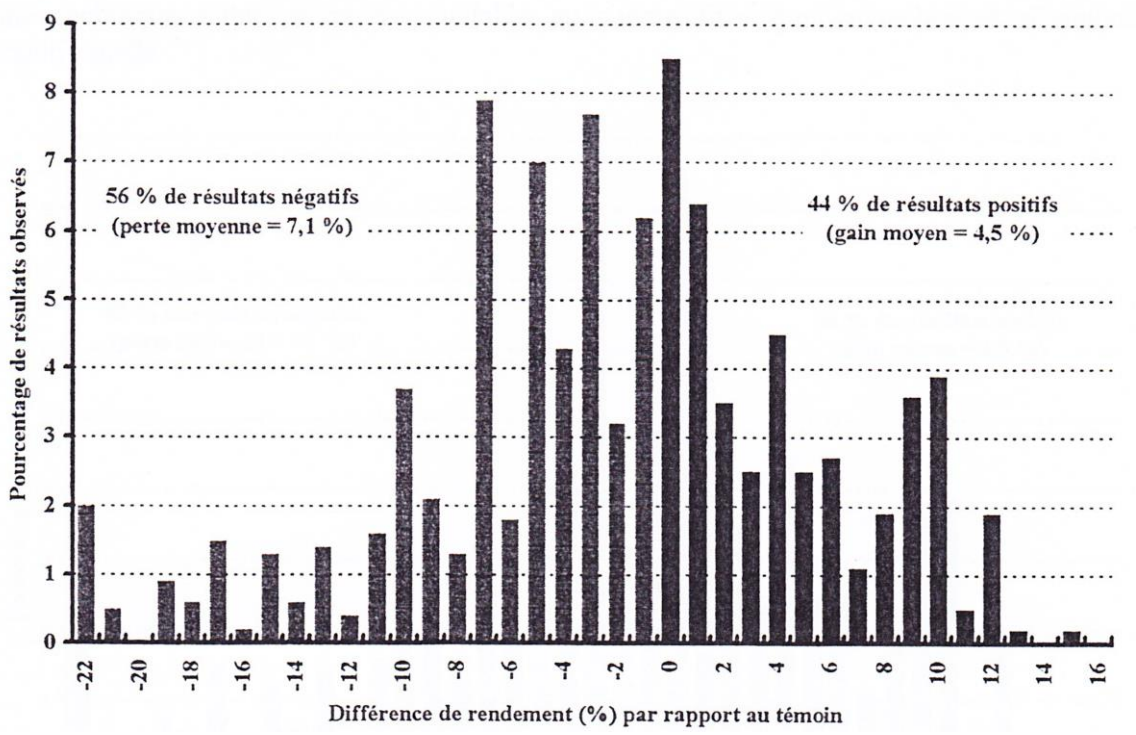


Figure 31 Percent difference (positive or negative) in sugar beet yield between reduced and conventional tillage (according to Roisin et al. (1997)).

The **soil texture** is also an important factor that determines the sensibility of soil to compaction. The major cereal-growing areas of Belgium are located in the upper part of Wallonia, characterized by a silty loam soil inherited from the loess deposit. Detailed analysis of the texture shows that this soil consists mainly of silt (70 to 80%) and considered to be light-textured. In general clay and silt-rich soils are more sensitive to soil compaction than sand-rich soils (Binkley and Fisher, 2012; Hillel, 1998). Clay-rich soils, however, have the ability to recover naturally through the shrink-swell processes, which is not the case with silt-rich soils. As a result, silt-rich soils located in wet climates, such as those found in Wallonia, are more subject to compaction and the recovery process can be very long in the absence of human intervention such as plowing. Problems related to soil compaction under no-till have been observed, for instance, in light-textured Danish soils subjected to wet conditions (Soane et al., 2012).

The **economic factor** is also significant in the success of conservation tillage implementation, as it determines the type of crops the farmer will introduce into crop rotation, and consequently the

type of soil tillage used. For example, in Wallonia the wheat crop is not profitable, and farmers introduce more profitable crops as much as possible, such as potato or sugar beet. But, as mentioned earlier, potatoes and sugar beet are soil structure-sensitive crops and conservation tillage practices are usually avoided.

As a result, the success of conservation tillage depend on a wide range of environmental, agronomic, economic, climatic and humans factors and cannot be generalizable in all agroecosystems worldwide.

Wallonia commences the exploration of soil microbiota

So far, technical limitations have led to neglecting the response of soil microbial communities to agricultural management practices in Wallonia. Not until the last decade have massive DNA-sequencing technologies made it possible to explore soil microbial diversity at higher resolution and throughput than were possible with previous DNA-based methods (e.g. fingerprinting) and culture-based approaches (Caporaso et al., 2011). The new technologies, however, require new expertise in handling and interpreting huge quantities of data with the help of appropriate bioinformatic (Gonzalez and Knight, 2012) and statistical tools (Buttigieg and Ramette, 2014). For this reason, their use is not yet widespread among university research teams. Although these new technologies still have limitations, they enable microbial ecologists to gain better understanding of changes in microbial diversity in space and time (Constancias et al., 2015; Fierer et al., 2009; Griffiths et al., 2011). This was thus a pioneering study in determining the responses of microbial diversity to different tillage regimes and crop residue management practices in the pedological and climate context of cropping systems in upper Wallonia.

The tillage regime affects chemical and physical soil conditions…

Previous measurements within the SOLRESIDUS experiment have shown both the soil structure and root system density to differ substantially between CT and RT. Under RT, the soil below 10 cm was much more resistant to the penetration (more than twice as much) than under CT, and the root system was confined to the first 10 cm. Under CT, the root system explored the whole soil profile (Eylenbosch et al., 2015). In addition, 6 years after conversion from CT to RT, we found the levels of some nutrients (e.g. phosphorus and nitrates), available carbon (HWC), and moisture to be lower than under CT. The tillage regime appeared also to affect the manner in which the soil conditions varied along the soil profile. RT-treated soil appeared having a more regular

horizontal stratification, CT-treated soil, having a less regular horizontal stratification[4]. One might expect these changes related to conversion to RT to have profound effects on the structure of microbial communities.

…thereby altering the structure of microbial communities

In our three studies, we clearly demonstrated substantial differences in α- and β-diversity between CT and RT, but we failed to observe any effect of crop residue management (Degrune et al., 2015 (Chapter III), 2016 (Chapter IV), 2017 (Chapter V)). Tillage-related changes in community structure appeared strongly related to tillage-regime-linked differences in physical and chemical soil properties, including moisture and nutrient status (Degrune et al., 2017 (Chapter V)), both of which were higher under CT. Regarding α-diversity, we found CT to result in higher richness and lower evenness than RT (Degrune et al., 2017 (Chapter V)). Our results should be interpreted with care, however, as our observations covered only the growing season (from March to June).

The quantity of crop residues is no big deal

It was surprising, at first glance, to observe no effect of crop residue management on α- and β-diversity. Recent studies (Ceja-Navarro et al., 2010; Navarro-Noya et al., 2013b) have found residue retention combined with zero tillage to increase the diversity and affect the structure of microbial communities. As reviewed thoroughly by Turmel et al. (2014), residue retention improves soil health and quality in many cases, thereby influencing the soil microbiota. In our study, however, the situation described as R- was not characterized by zero crop residues, but by retention of a lesser quantity of crop residues than in the situation described as R+. Therefore, crop residues influenced the physical, chemical and biological soil components in both cases. What we assumed was that the additional quantity of crop residues present under R+ conditions did not provide any extra benefits in terms of soil health and quality, and hence did not affect soil microbial diversity.

Another important factor to consider is the duration of the experiment. We view this to be relatively recent (6 years), so the effect of crop residue quantity on soil quality might not yet be observable.

[4] An additional analysis was performed on the basis of Degrune et al. (2016). We determined the depth effect under CT and RT. Under CT, the depth effect was weaker (t=1.4, P=0.07) than under RT (t=2.5, P=0.0002).

The Shannon dark side

A slight but significant effect of tillage regime on α-diversity was noted (Degrune et al., 2015 (Chapter III), 2016 (Chapter IV), 2017 (Chapter V)). We used three different indexes to evaluate α-diversity: OTU richness (S), based on the number of different OTUs, and two indexes reflecting evenness, i. e. equitability (or distribution) of relative OTU abundances: the Shannon index (H) and the Smith-Wilson (SW) index. Eveness establishes whether a community is dominated by a few OTUs (**Figure 32a**) or whether OTUs are equally distributed (**Figure 32b**).

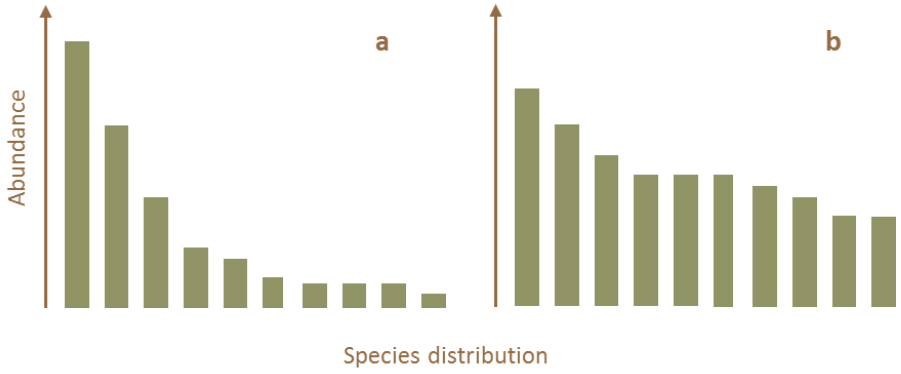


Figure 32 Schematic representation of OTU distribution in the microbial community. Left: the community is dominated by a few OTUs (low evenness), right: the OTUs are more equally distributed (high evenness), the number of OTUs being the same in both cases.

On the basis of the different diversity index values recorded in CT- and RT-treated soil (summarized in **Figure 33**) , we have clearly shown the indexes H and SW to vary in opposite directions.

H is the most common diversity index used in ecology as it combines richness and eveness in one single index. However, the dependence of H to richness must be considered when assessing eveness to avoid a misleading interpretation. A robust approach for accurately assessing eveness might be to use another metric, such as SW, which is totally independent of richness.

To date, however, although many diversity metrics are available, no consensus has emerged on an exact definition of evenness (Tuomisto, 2012). Moreover, it is important to note that bulk parameters such as α-diversity metrics are likely to be insufficient for capturing structural changes in complex communities (Hartmann and Widmer, 2006).

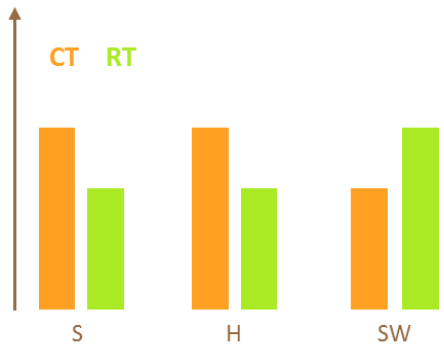


Figure 33 Schematic representation of α-diversity index values found in our study under CT (conventional tillage) and RT (reduced tillage). OTU richness (S) reflects the number of different OTUs, while both Shannon (H) and Smith-Wilson (SW) reflect OTU eveness. H combined richness and eveness, while SW is richness independent and reflect eveness only.

CT-treated soil is species richer than RT-treated soil, but less even

We found CT-treated soil to be species richer, less even than RT-treated soil. On the basis of the intermediate disturbance hypothesis (IDH) or "hump-back model" describing the response of a community to stress (Giller et al., 1998), one might assume conventional plowing to act as an intermediate disturbance which is neither too rare nor too frequent, and to result in maximization of OTU diversity. Plowing mixes the different horizons and breaks down soil aggregates, which in turn release organic matter, thus creating new ecological niches allowing colonization by minor or new species (Tilman, 1982). The disturbance might favour microorganisms featuring an r-strategy, thus leading the community to be dominated by a few OTUs with high competitive ability. This would result in lower evenness than under RT. Yet as our estimate of diversity was based on a specific growing season (March to June), we cannot assert that this conclusion holds throughout the year. To see if it does, it will be necessary to perform analyses based on a whole one-year period.

Microbial responses depend on depth and growing season

We have found the effect of the tillage regime on microbial community diversity to be influenced strongly by the soil depth (Degrune et al., 2016) and moderately by the growing season (Degrune et al., 2017). Bacterial and fungal communities showed different patterns of variation in response to these factors.

That bacterial and fungal communities responded differently to depth might be due to the fact that these organisms are not influenced by the same drivers. The pH is well known to have a major

influence on bacterial diversity (Lauber et al., 2009), while fungal diversity is more strongly affected by changes in nutrient content (Leff et al., 2015). Soil moisture has also been identified as an important determinant of microbial diversity (Brockett et al., 2012). In our study these factors varied, in some degree, with depth and tillage regime. In addition, previous measurements of root density (Eylenbosch et al., 2015) have shown it to vary between CT and RT in relation to depth. This might also explain why bacterial and fungal communities showed different patterns of variation between CT and RT, as the activity of the root system is likely to influence bacterial and fungal diversity (Philippot et al., 2013a).

A moderate interaction of tillage regime with growing season was also observed (Degrune et al., 2017 (Chapter V)). The establishment of the root system was expected to "dilute" the tillage regime effect, as the root system significantly influences the surrounding soil and may lead, for example, to changes in the carbon source (root exudation), pH (ion release or uptake), water and oxygen contents (root water uptake and respiration), and nutrient availability (plant uptake and secretion of chelators that sequester micronutrients) (Philippot et al., 2013a). Soil structure, which determines pore size and connectivity and the associated fluxes of oxygen and water, is also influenced by the root system (Bronick and Lal, 2005). Lastly, climate conditions (e.g. temperature, moisture), which change over the growing season, are liable to contribute to the interaction between tillage regime and growth season.

Our observations verify our hypothesis, i.e. difference between microbial communities in CT- and RT-treated soils would diminish over time, but the interaction between tillage regime and growing season was less pronounced than expected. The sampling design of our study might provide an explanation. The samples were collected as close as possible to the stem, so that the soil collected was neither totally bulk soil nor totally rhizosphere. We assume that a greater effect of the growing season might be detected if samples were collected within the rhizosphere, i.e. the narrow region of soil that is directly influenced by root secretions.

What are the ecological meanings of our findings?

Increased richness does not ensure "happiness"

Our results related to α-diversity raise important questions regarding its ecological consequences for ecosystem functioning and thus crop productivity. According to current understanding, higher levels of species richness generally correspond to increased ecosystem functioning, but the magnitude of this effect remains to be further explored (Hooper et al., 2005; Ricketts et al., 2016). Ecosystem functioning related to organic matter transformation and nutrient cycling depends on soil processes mediated largely by microorganisms (East, 2013; Nannipieri et al., 2003; van der Heijden et al., 2008). Yet some processes (e.g. carbon mineralization), called "broad" processes by Schimel et al. (2012), are mediated by a large group of diverse microorganisms, whereas others (e.g. nitrification) are mediated by only a few specific soil microorganisms. Schimel et al. (2012) call these processes "narrow" processes. Alpha-diversity alone is thus not a good predictor of ecosystem functioning, as "keystone" species (abundant or rare) can strongly influence certain soil processes. Consequently, we have employed recent DNA-based methods allowing identification of such taxa and their changes in relative abundance between CT and RT.

Capturing reality is not that simple: the art of telling the story

Exploring the structure of microbial communities in a complex environment such as soil is now possible with recent advances in high-throughput sequencing (Cardenas and Tiedje, 2008). In our study, we used 454 pyrosequencing technology to infer community structure in relation to tillage regimes associated with different crop residue management practices.

Given the huge number of microbial species present in the environment and the fact that microbial ecologists are still struggling with species definitions, it remains difficult to infer community structure at the finest level of organization. Consequently, OTU-based methods (where all sequences are clustered into OTUs on the basis of their similarity at a certain threshold - usually 3% dissimilarity) are commonly used to infer the structure of microbial communities in diverse environments such as soil (Chen et al., 2013). Yet in contrast to plant and animal species, which can be associated with ecological meanings, microbial species remain ill defined, especially since so many organisms have never been cultured or characterized. If one wishes to associate OTUs with ecological meanings in order to predict ecosystem functioning, it is therefore more appropriate to

examine community structure at a higher taxonomic level (from phylum to genus) (Philippot et al., 2010).

In the most recent studies having used high-resolution sequencing methods to explore community structure in relation to different soil management practices, the phylum and class levels appear to have been the most used (Carbonetto et al., 2014; Dorr de Quadros et al., 2012; Jiménez-Bueno et al., 2016; Navarro-Noya et al., 2013a; Sengupta and Dick, 2015). This approach fails to tell the whole story. As previously shown in Degrune et al. (2015) (Chapter III), an overall response to tillage at phylum level may mask patterns that can only be viewed at a lower taxonomic level, and when no effect of tillage is observed at phylum level, this could be because different sub-groups have opposite responses to tillage. It is therefore important to infer community structure at different levels of taxonomic resolution, since conserved traits – which can lead to functions – can be shared among taxonomic levels (Martiny et al., 2013, 2015).

Since investigators are still working on ways to infer community structure at multiple taxonomic resolutions, we have developed a method that exploits the obtainable dataset so as to discern patterns at different taxonomic resolutions (Degrune et al., 2015 (Chapter III)). Our method can identify tillage-sensitive taxa at any level, but it requires adequate data visualization tools, such as taxonomic trees, to get a general view of microbial taxonomic diversity (Hartmann et al., 2015; Degrune et al., 2017 (Chapter V)). The taxonomic tree of **Figure 29**, Chapter V proved to be a practical tool both for getting a direct overview of community diversity and for distinguishing, at different levels of organization, variations in community structure related to the tillage regime. It is also possible to display the abundance of each OTU and associated statistical tests. On the basis of such analyses we can start to speculate, taking into account the measured soil parameters, as to why some taxa showed a higher relative abundance under CT and others under RT.

Soil microorganisms have habitat preferences

We have clearly identified differences between CT- and RT-treated soils in terms of soil β-diversity. We have attributed these differences to changes in edaphic properties, including moisture and nutrient content (**Figure 27**, Chapter V) (Degrune et al., 2017). Among others, moisture (Brockett et al., 2012), nutrient content (Leff et al., 2015) and oxygenation (Hartmann et al., 2014; Lüdemann et al., 2000) are recognized as important determinants of microbial community structure.

Although the observed effect of the tillage regime on microbial diversity is consistent with the results of recent studies based on high-resolution techniques (Carbonetto et al., 2014; Dorr de Quadros et al., 2012; Jiménez-Bueno et al., 2016; Navarro-Noya et al., 2013a; Sengupta and Dick, 2015), the direction of change of some microbial groups was not always consistent with reported results. For example, Carbonetto et al. (2014) report a higher relative abundance of Actinobacteria in no-tilled soil and a higher relative abundance of Nitrospirae in tilled soil. We, in contrast, evidenced a higher relative abundance of Actinobacteria and a lower relative abundance of Nitrospirae under CT than under RT (**Figure 28**, chapter V).

Hence, although there is a consensus that tillage alters soil microbial community structure, the response of an individual group appears to be very context specific and cannot be generalized across various agroecosystems. The response of the soil microbiota depends largely on the physical and chemical conditions caused by soil tillage system, which in turn differ according to a wide range of parameters as mentioned earlier, such as soil type, soil moisture and structural conditions occurring at the time of soil tillage, and the type of machinery and tools used.

Our detailed assessment of taxonomic groups has enabled us to identify tillage-sensitive microbial taxa and speculate on the reason why these taxa were more abundant under CT or RT (Degrune et al., 2017).

Among them, some have lifestyles that may shed some light on their higher abundance under a given tillage regime. This applies notably to the effect of tillage regime on the relative abundances of bacteria having an oligotrophic[5] or a copiotrophic lifestyle[6] strategy (Fierer et al., 2007a). We also found the taxon Glomeromycota, containing the well-known ecologically and economically important AMF (van der Heijden et al., 2008), to be more abundant under RT, believed to favour a less nutrient-rich soil than CT. This group is of particular interest as an indicator of nutrient-poor soil conditions (Leff et al., 2015). We also found under RT a greater abundance of several groups of the recently suggested candidate phyla radiation (CPR) (Brown et al., 2015). These groups feature lifestyles (Nelson and Stegen, 2015; Yeoh et al., 2015) that appear to favour adaptation to more nutrient-poor conditions. Other taxa, capable of degrading more complex and recalcitrant compounds such as lignin (commonly found in crop residues), appeared more abundant under CT.

[5] Microbes thriving better under low nutrient availability.
[6] Microbes thriving better under high nutrient availability.

Finally, the greater soil compaction found under RT might explain the higher occurrence of taxa previously observed in oxygen-limited environments, including Nitrospirae (Hartmann et al., 2014) and Clostridiales members (Goldfarb et al., 2011).

However, our considerations on the interactions between soil microbiota and its surroundings remain purely speculative as based on the literature, i.e. if these microorganisms have been previously found in a specific environment (oxygen-limited, nutrient-limited, etc.), or if they have been cultured and their physiology and metabolism determined. Moreover, our study found no evidence that these tillage-sensitive taxa were actually active in delivering ecosystem functions.

In addition to the physical and chemical factors, other factors, such as the interactions of soil microbes with plants, are likely to modify the structure of soil microbial communities (Prober et al., 2015). These plant-microbiome interactions are of particular importance in the frame of developing sustainable agriculture (Barea, 2015; Trognitz et al., 2016).

Even if the recent metabarcoding techniques have permitted to gain knowledge in the structure of the soil microbiota, a large number of soil microbes remain largely unexplored regarding their metabolic capabilities and ecological roles. For example, even if very diverse and widespread in soils, very little is known about Acidobacteria members because they are poorly represented in soil culture collections. Another salient example is a recent candidate phylum radiation. So far, this new group has been poorly explored, but it has appeared to be highly diverse in the recent tree of life (Hug et al., 2016).

Methodological limitations

The methodology we used to explore the structure of microbial communities has pitfalls and limitations that must be considered to avoid drawing incorrect and misleading conclusions. Below we discuss the main errors that can occur at different steps of data analysis, including field-based and technique-based errors (**Figure 34**).

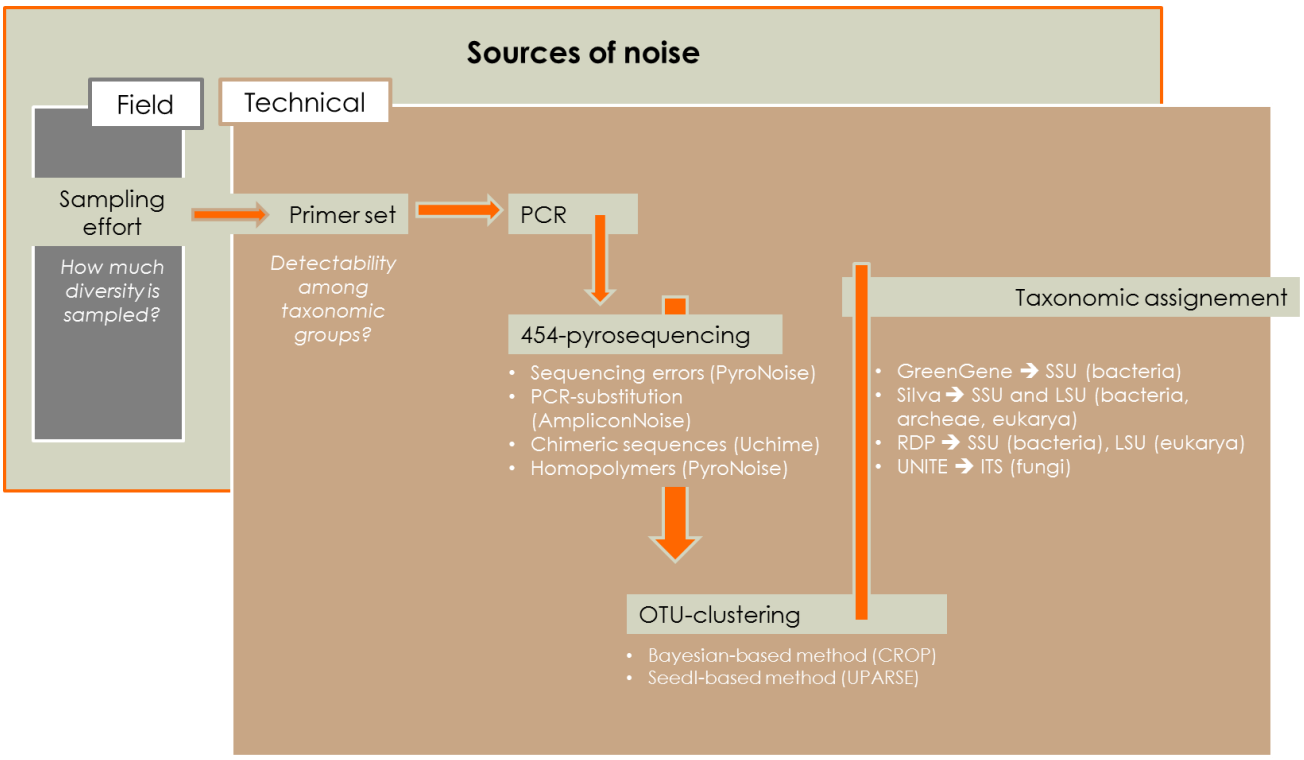


Figure 34 Schematic representation of field-based and technique-based pitfalls and limitations of the method to explore the soil microbial diversity.

Field-based

Among the major sources of **field-based errors** is the sampling design, as it largely determines our ability to capture enough OTU diversity to draw robust conclusions. The different treatments (RT/R+, RT/R-, CT/R+ and CT/R-) were replicated four times in a Latin square (plot replicates). Therefore, we used a rarefaction curve (Hughes et al., 2001) to evaluate the sampling effort and to see if most of the OTU diversity was captured with four plot replicates. We estimated, on the basis of the shape of the curve (**Figure 21**, chapter IV), that most of the OTU diversity was covered. By increasing the number of samples one could increase the detectability of rare OTUs, since singleton OTUs, (i.e. OTUs represented by a single sequence), were considered sequencing errors and discarded.

Technical-based

Technique-based errors result mostly from the primer set chosen to amplify the target population and from PCR amplification, pyrosequencing, the method chosen to cluster sequences into OTUs, and the database chosen to assign the sequences. We considered these sources of bias to be the major ones, and we do not discuss here any other potential sources of technical bias, such as DNA extraction, sample preparation, PCR conditions, and the various sequencing and bioinformatics protocols available.

- We used 454 pyrosequencing technology, an amplicon-based sequencing method based on the use of genetic markers to amplify a region of the genome. The choice of the region to be amplified, and hence of the universal primer set to be used, is crucial: according to the region selected, some taxa will be more readily detected than others (Ghyselinck et al., 2013). Thus, the probability of detection is not the same for all OTUs. To address this issue, one must use online tools such as TestPrime on the SILVA platform (Quast et al., 2012) to check the set of primers in order to validate their coverage of the diversity and to make sure all taxonomic groups are well represented.
- **PCR-** and **platform-specific errors** also occur and lead to inflating the number of OTUs, i.e. overestimating the diversity of microbial communities. Bioinformatics tools are now available for processing the raw data and removing as many errors as possible. Among the tools for processing pyrosequencing data, the AmpliconNoise algorithms are used to remove **PCR-substitution** errors (Quince et al., 2011). **Chimeric sequences** - composed of two true microbial sequences wrongly assembled – can be removed with the UCHIME algorithm (Edgar et al., 2011), and correction for both **homopolymers** – not accurately detected with pyrosequencing technology – and **sequencing errors** can be done with the PyroNoise algorithm (Quince et al., 2009). Finally, inappropriate OTU clustering can lead to an incorrect estimate of microbial diversity. In our study, we used **Bayesian** clustering with the CROP algorithm (Hao et al., 2011), which is robust against sequencing errors and produces more accurate results than conventional clustering methods. However, this time-consuming and computer-intensive tool cannot be used with large datasets such as those produced by Illumina MISeq technology. In such cases**, seed-based** clustering algorithm such as UPARSE must be preferred (Edgar, 2013).

- Concerning **taxonomic assignment**, since sequencing the ribosomal RNA (rRNA) gene became widely used in microbial surveys to explore bacterial and fungal diversity, several sub-databases (derived from the main ones), have been developed to provide quality rRNA sequences of reference for phylogenetic and taxonomic classification. Among the most employed databases, GreenGene (http://greengenes.secondgenome.com/downloads) is dedicated to the gene encoding small-subunit 16S rRNA, SILVA (https://www.arb-silva.de) provides both small-subunit and large-subunit sequences from all three domains of life (16S and 23S sequences for Bacteria and Archaea; 18S and 28S sequences for Eukarya), RDP (https://rdp.cme.msu.edu/) provides bacterial and archaeal small-subunit sequences (16S rRNA) and fungal large-subunit sequences (28S rRNA), and Unite (https://unite.ut.ee/) is dedicated solely to providing reference sequences from the ITS region of fungi. Each of these databases possesses its own classification and annotations that can differ from those of other databases and hence lead to different conclusions.

At this time, no reference pipeline is available for processing microbial data from high-throughput sequencing. Users either employ a ready-to-use pipeline such as mothur or RDP, or customize their own pipeline and integrate multiple tools according to their own expertise (Gonzalez and Knight, 2012).

In our study, importantly, these sources of error were of little importance, since we compared samples subjected to the same field-based and technique-based errors. In addition, although our protocol for processing raw data evolved, each research question (associated with Chapter III, IV, or V) was analysed consistently according to the same protocol.

It is also worth mentioning that even though 454 technology has been widely used over the last decade in soil microbial surveys (e.g. Buee et al., 2009; Degrune et al., 2016; Hartmann et al., 2015, 2014; Lauber et al., 2009; Zhang et al., 2012), the Roche Company has recently announced the withdrawal of the 454 pyrosequencing platforms by 2016. To date, Illumina MiSeq technology has already been widely applied for soil microbial studies and features a good cost-efficiency (D'Amore et al., 2016). This technology was already used in the frame of the Farm4 Future project (see *prospects and improvements*).

2. Agronomic conclusion

Our study has provided a new understanding of improving the sustainability of agroecosystems in Wallonia. Given the important role of soil microbiota in maintaining soil functions, our study unravelled the microbial taxonomic diversity in the Walloon cropping system. It also provided a better understanding of the response of microbes to different soil tillage and crop residue managements in relation to changes in the physical and chemical properties of soil at different soil depths and growing seasons.

Among our main findings, we highlighted the fact that soil under conventional tillage (plowing) was not associated with a loss in microbial diversity, and that reduced tillage can lead to severe soil compaction that in turn may adversely affect soil quality.

Our results can have major agronomic consequences regarding the reform of the common agricultural policy (CAP) in Europe. To date, in Europe, even if the transition to conservation soil tillage practices may offer a number of economic and environmental advantages compared to plowing, these practices still rely on the utilization of phytosanitary products to control weeds and pests. Despite the disadvantages for soil quality of systematic plowing, it remains an effective practice for controlling pests and weeds. Therefore, plowing makes it possible to limit the use of phytosanitary products. From this perspective, and in line with the near future strict regulation of agro-chemical products from the CAP, new conservation practices should be designed. We would recommend that plowing is not systematically excluded but instead used occasionally and smartly. This would overcome the disadvantages of both conventional and conservation practices.

Finally, it is worth mentioning that our results are not generalizable to other agroecosystems, even if located in the same geographical area. With respect to the wide range of factors (e.g. edaphic, climatic, technical, economic, etc.) that can influence the success of implementation of soil conservation practices, it is clear that further studies are needed to better understand how to implement these practices and design them so that they fit with local context, while improving the sustainability of these agroecosystems.

3. Prospects and improvements

Using high-resolution taxonomic analysis of largely unexplored soil microbial communities, we have gained an idea of changes in the structure of microbial communities associated with different soil management practices. However, our study was context specific and given the wide range of factors that can influence the outputs, our results are not generalizable to other agroecosystems. Besides the edaphic and climatic context, there are other factors that are not easy to control, such as the type of machinery and tools used, the duration of the experiment, the choice of phytosanitary products (a wide range of products are available), the tillage period (e.g. soil humidity conditions) and the history of soil management, all of which may contribute to contrasting results from different studies worldwide.

Although the control of all factors is impossible, we suggest some improvements and other approaches to achieve a deeper understanding of the soil microbiota response to soil management practices.

Increasing the number of observations

Here, we did not test the spatial variability of our results because only one observation (i.e. one sample=composite of sub samples) was made per plot. Moreover, it is worth mentioning that the sample collection was performed in a delimited area (around 6m2). Given the spatial variability of soil chemical properties (see **Figure 4 to 9**, chapter I), we suggest to increase the number of observations to gain a better understanding of the spatial variability in soil. Thus, the reliability of the results will be increased.

Increasing the observation period

We strongly advise extending the observation period to the entire year. Indeed, our study is focused on a few observations performed during the growing season (4-5 months). The observations should be replicated over several years to take into account the variability of factors such as climate and crop rotation.

Enlarged to different soil texture conditions

We suggest replicating the experiment in different edaphic contexts to take into consideration the large diversity of soil found in Wallonia. Based on the soil map of Wallonia (**Figure 35**), the diversity of soil texture found in the cropping area in upper Wallonia is wide and can result in contrasted soil responses to tillage, and thus a different response of the soil microbial community diversity. More importantly, the soil type and texture have been shown to drive substantially the structure of soil microbial communities (e.g. Bach et al., 2010; Chau et al., 2011; Ulrich and Becker, 2006).

Importantly, the impact of tillage practices on the quantity and quality of organic matter has been recognized (Six et al., 1999, 2000), and this impact seems to vary with the pedological context as demonstrated in preliminary results from an ongoing project Farms4Future (**Box 3**). In this study, we evidenced different dynamics of organic carbon associated with different farming systems (organic vs. conventional) in contrasted soil texture conditions (silty and sandy) (Tullii, 2016). The impact of the farming system on organic C concentrations (**Figure 36**) and quality (results not shown) differs in relation with the soil texture.

Box 3 – Farms4Future in brief

Farms4Future is an ongoing FNRS-funded project started in 2015. Its aim is to compare the performances of Walloon agroecosystems featuring different farming systems (conventional and agroecological) in terms of crop productivity and environmental and social costs. The performances are evaluated in contrasting pedological contexts.

In the same study Farms4Future, we showed that the magnitude of the effects of farming on the microbial community structure differs according to the different soil textures (**Figure 37**).

Since the soil microbiota is influenced by the quantity and quality of organic C (Eilers et al., 2010; Kameshwar and Qin, 2016), as well as by the soil texture (Mummey et al., 2006; Sessitsch et al., 2001), the investigating of soil treatment effects under different soil textures is of major interest and requires further studies in Wallonia.

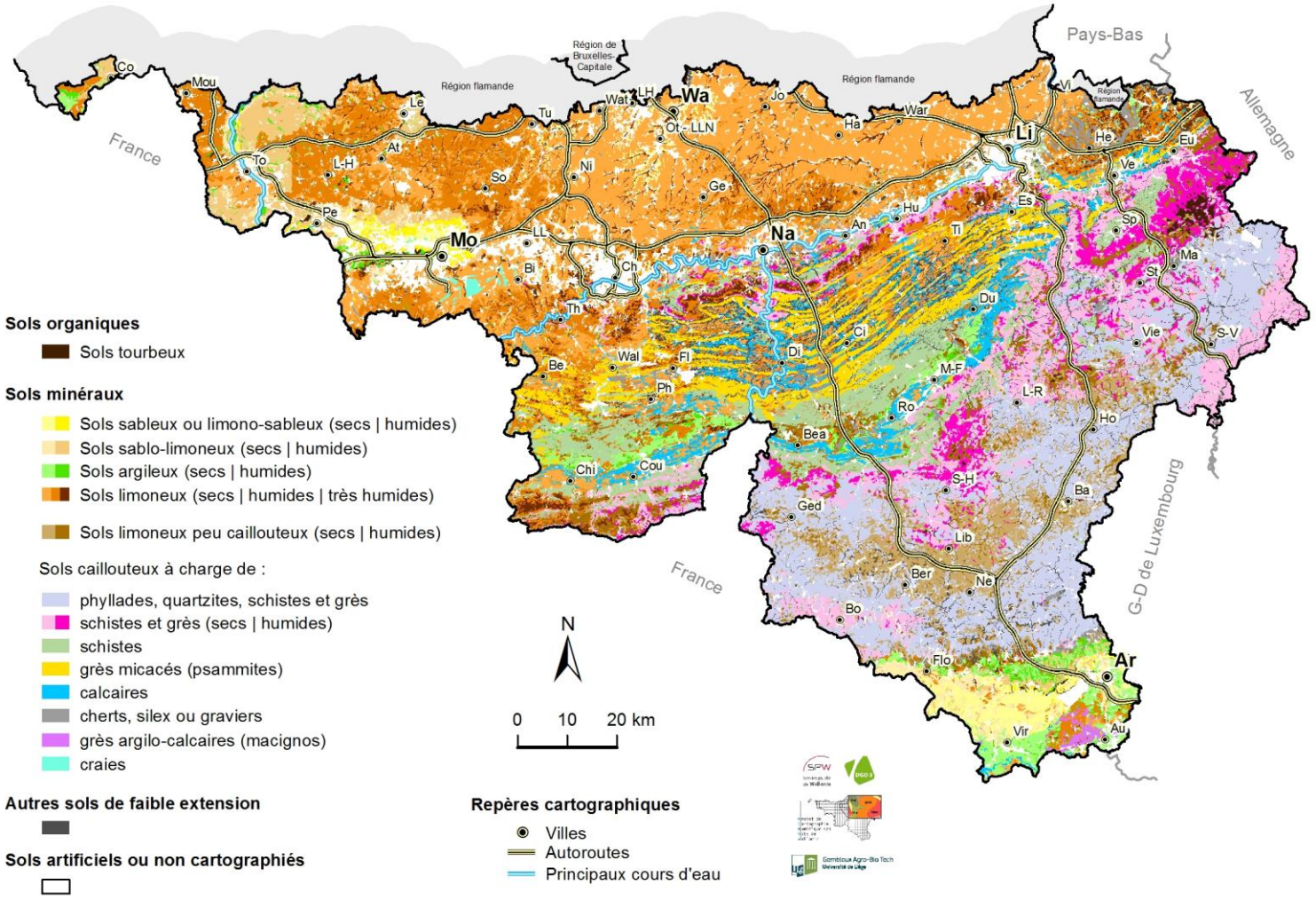


Figure 35 Map of the Walloon soil types (in french). The soil in the upper part is highly fertile (orange area) and widely used for cropping. Within this area, there is a wide range of different soil textures. *Source: Bock et al. (2006)*.

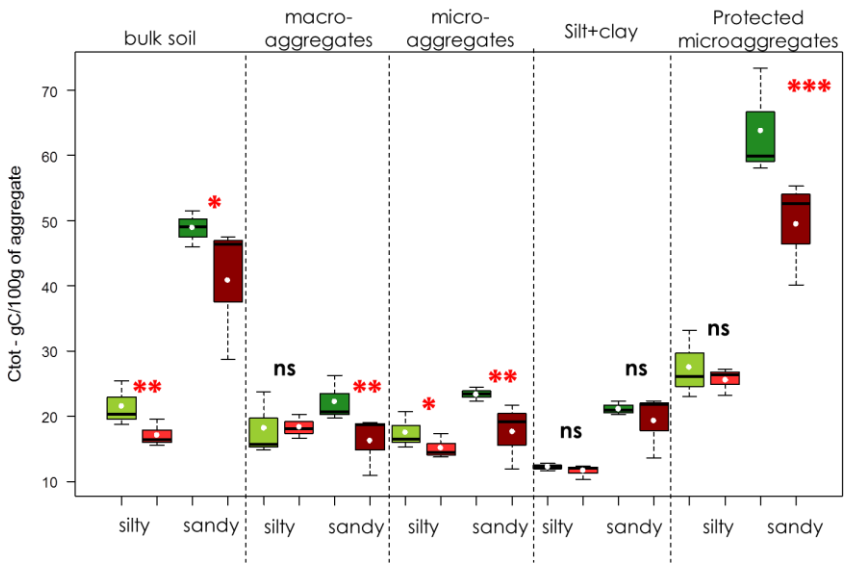


Figure 36 Boxplot representation (n=9) of organic carbon concentration in different size class of aggregates, and in relation to different farming systems: green=organic and red=conventional, under contrasted textural soil (silty and sandy). The magnitude of the impact of the farming system on the C concentration might differ according to the soil texture.

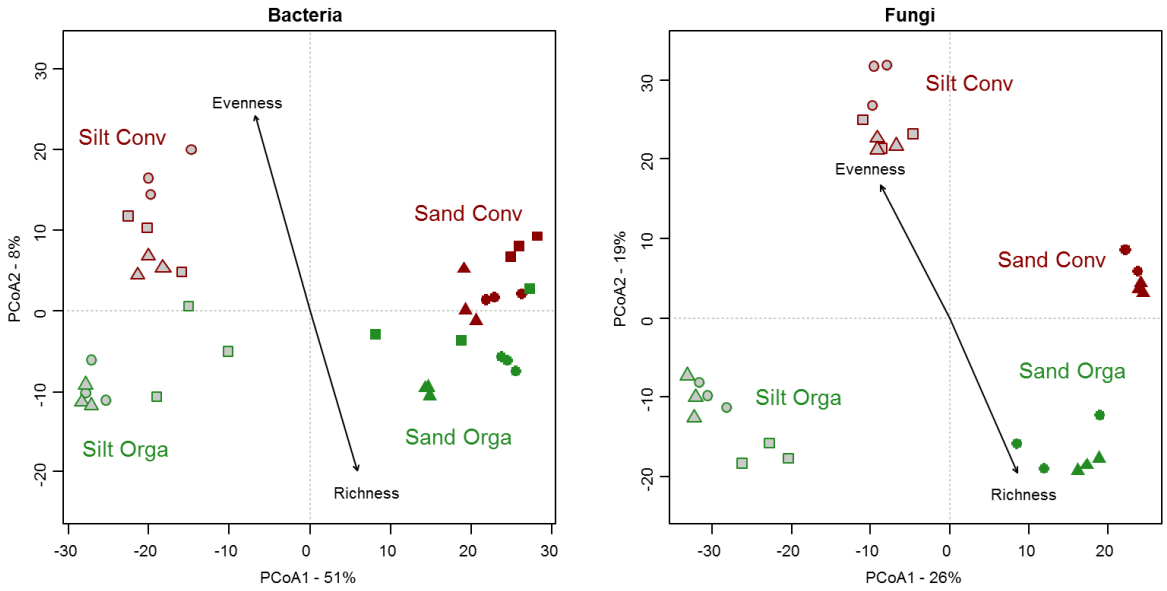


Figure 37 Farms4Future preliminary results on the effects of the farming system and soil texture on bacterial and fungal β-diversity. On the basis of distances among groups, the farming system effect appears larger for silt samples than for sand samples. The farming system is represented by a color code with conventional=red and organic=green and the soil texture is represented by the colour used to fill the symbol, with silt=grey and sand=coloured.

Gaining knowledge with complementary approaches

Capturing the active fraction of soil microbes

Soil microbial-based processes are mediated by the active fraction of microorganisms, which is commonly 2.5 to 5 times lower than the inactive fraction (e.g. dormant cells) (Blagodatskaya and Kuzyakov, 2013). Our approach is based on DNA, universally present in both active and inactive microorganisms. Hence, to explore the microbial diversity part that actively contributes to the functioning of the soil ecosystem, additional complementary approaches are required. The wide range of techniques available, as well as the associated benefits and trade-offs are well reviewed in Blagodatskaya et Kuzyakov (2013). A brief overview is provided in **Figure 38**.

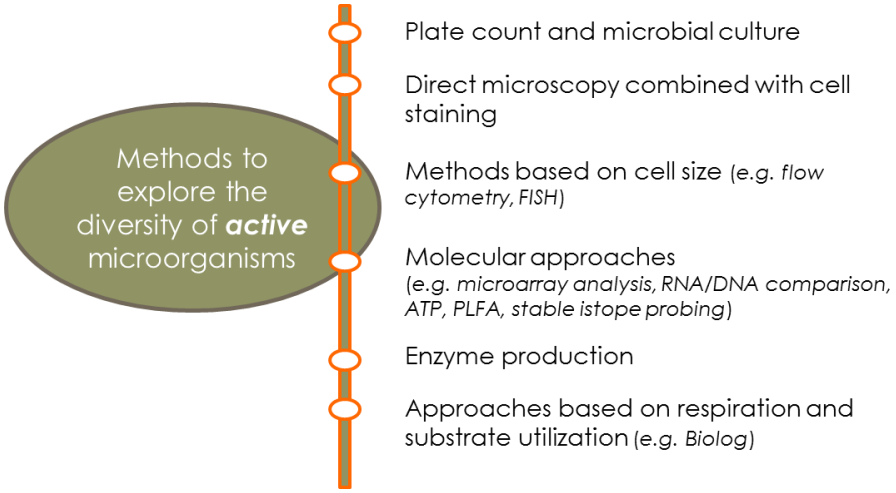


Figure 38 Brief overview of available methods to explore the diversity of active microorganisms (according to Blagodatskaya and Kuzyakov (2013)).

Trait-based approach

Our study has provided no evidence of whether the observed differences in microbial diversity between CT- and RT-treated soils influence soil processes liable to affect agroecosystem functioning and crop productivity. On the basis of our taxonomic results, we have exploited current knowledge from the literature to speculate on the lifestyles, feeding types, or ecological roles of some taxonomic groups. Originally these lifestyles, etc. were observed because most members of the selected group share ecological traits and therefore respond similarly to environmental variations. But in many cases, ecological traits are less conserved and more scattered across the

taxonomic tree (Martiny et al., 2013, 2015), making it very hard to deduce functions from a taxonomic approach.

Recently, trait-based approaches have emerged to provide a mechanistic understanding of the role of biodiversity in maintaining multiple ecosystem processes and services (Krause et al., 2014; McGill et al., 2006). These approaches focus on the functional characteristics (or traits) of individuals that in turn may contribute to the delivery of ecological processes and functions. Traits refer to the physiology, morphology, or genomic characteristics that affect the fitness or function of an organism (Violle et al., 2007).

Although recent studies have used trait-based approaches to predict a number of functions based on different methods (e.g. Allison, 2012; Wieder et al., 2015), it remains hard to determine which microbial traits it is important to measure in relation to ecosystem functioning, and how to measure them. These challenges notwithstanding, a number of tools are already available for use in studies investigating relationships between biodiversity, ecosystems, and their functioning (Krause et al., 2014) (**Figure 39**).

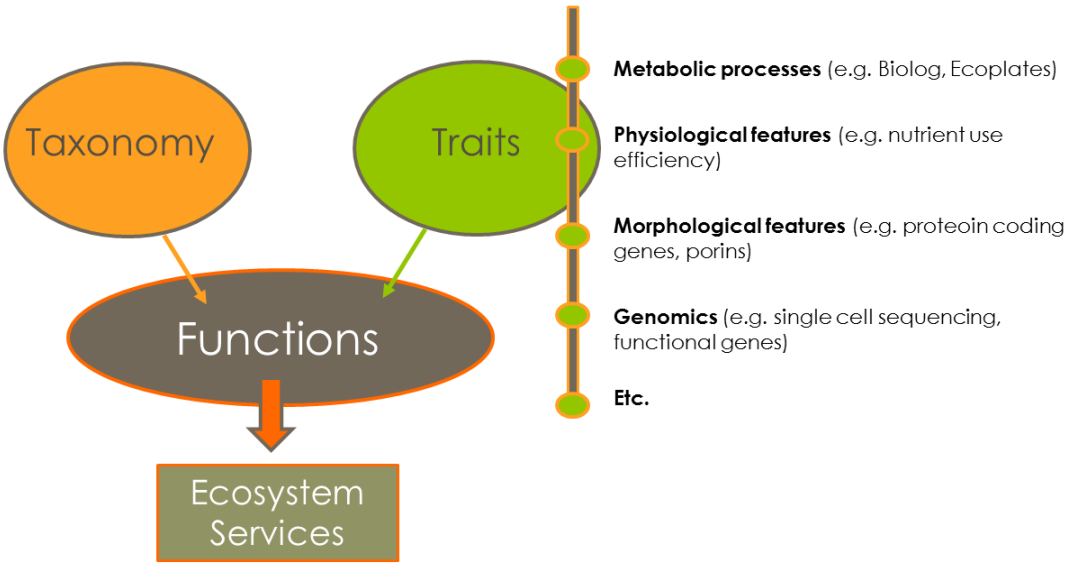


Figure 39 Schematic representation of the two approaches (taxonomy and traits) to linking microbial community structure to functions and finally ecosystem services.

Agronomic perspectives

The controlled SOLRESIDUS experiment has enabled us to isolate the effects of two factors (tillage regime and quantity of crop residues) on microbial diversity. As next step, a more holistic approach might be used to analyse responses of microbial diversity on emergent properties resulting from the action of a wide range interacting factors. Farms4Future is an example of a project using a holistic approach. Its aim is not to isolate the effect of one or two factors, but rather to compare the overall performances of conventional and agroecological agroecosystems by assessing the diversity of ecosystem services delivered. With a holistic approach, we examine agroecosystem performance as it results from the effects of emergent properties, while with a controlled approach, we examine the specific effects of one or two isolated factors (**Figure 40**).

The controlled and holistic approaches are complementary, but given the new and urgent challenges the agriculture of tomorrow has to face, it is time to devote experiments to investigating the performance of agroecosystems, laying temporarily aside the question of which factors produce which effects.

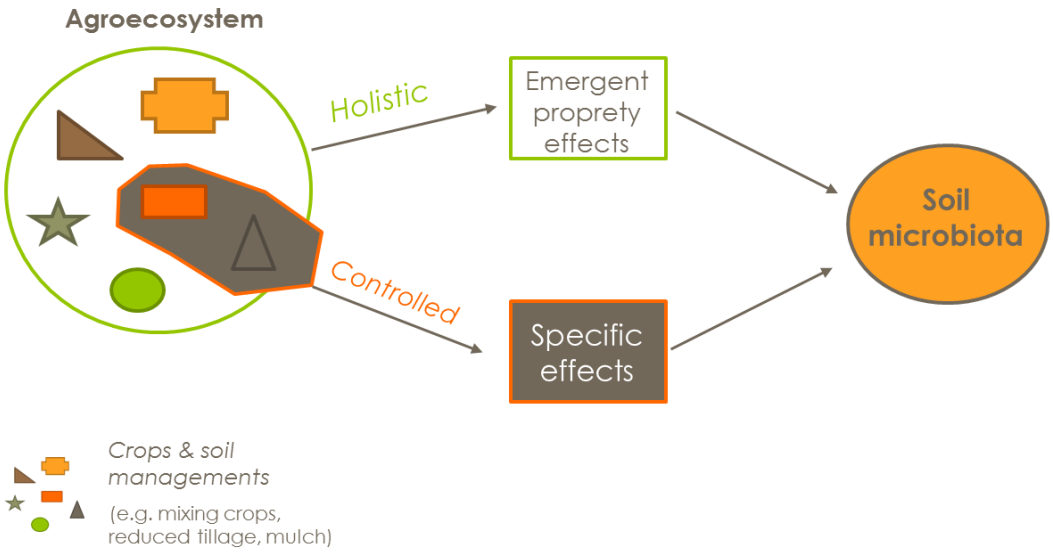


Figure 40 Schematic representation of two different and complementary approaches, holistic and controlled, used to infer microbial diversity in agroecosystems

4. Closing words

Over the last four years of the project, our perception of soil microorganisms in relation to soil management has profoundly changed. In the context of the fertile soils found in Wallonia, our study was a pioneer in getting insights into soil microbial diversity at community level, using novel high-throughput sequencing technology. In Gembloux Agro-Bio Tech, before the start of the AgricultureIsLife project in 2013, the soil microbiota was explored by means of very low-resolution methods enabling us to detect only a narrow part of the diversity, comprising specific microbial groups representing less than 1% of the total diversity. With our study, we have highlighted the complexity of relationships between the unexpectedly high microbial diversity and soil management practices.

Having reached the end of our project, we have answered some questions but we have also raised new ones, notably regarding the functional roles of soil microorganisms in agroecosystems. In addition, we have focused here on microbes, but future research should take into account also the other organisms that live in the soil, which are very important for soil functioning and which interact with each other and with members of microbial communities.

Our study has led us to ‘play’ with various disciplines to answer our different questions: agronomy, microbial ecology, molecular biology, bioinformatics, and statistics. We point out that more holistic and multidisciplinary approaches are now required to increase our understanding of agroecosystem functioning with a view to achieving sustainability.

5. References

Allison, S.D., 2012. A trait-based approach for modelling microbial litter decomposition. Ecol. Lett. 15, 1058–1070. doi:10.1111/j.1461-0248.2012.01807.x

Bach, E.M., Baer, S.G., Meyer, C.K., Six, J., 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. Soil Biol. Biochem. 42, 2182–2191.

Barea, J.M., 2015. Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. J. Soil Sci. Plant Nutr. 15, 261–282. doi:10.4067/S0718-95162015005000021

Basch, G., Friedrich, T., Kassam, A., Gonzalez-Sanchez, E., 2015. Conservation Agriculture in Europe, in: Farooq, M., Siddique, K.H.M. (Eds.), Conservation Agriculture. Springer International Publishing, Cham, pp. 357–389.

Binkley, D., Fisher, R., 2012. Ecology and management of forest soils. John Wiley & Sons.

Blagodatskaya, E., Kuzyakov, Y., 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biol. Biochem. 67, 192–211.

Bock, L., Bah, B., Veron, P., Lejeune, P., 2006. Carte des Principaux Types de Sols de Wallonie à 1/250 000.

Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biol. Biochem. 44, 9–20. doi:10.1016/j.soilbio.2011.09.003

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3–22.

Brown, C.T., Hug, L.A., Thomas, B.C., Sharon, I., Castelle, C.J., Singh, A., Wilkins, M.J., Wrighton, K.C., Williams, K.H., Banfield, J.F., 2015. Unusual biology across a group comprising more than 15% of domain Bacteria. Nature 523, 208–211. doi:10.1038/nature14486

Buee, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S., Martin, F., 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol. 184, 449–456.

Buttigieg, P.L., Ramette, A., 2014. A guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses. FEMS Microbiol. Ecol. 90, 543–550. doi:10.1111/1574-6941.12437

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. 108, 4516–4522.

Carbonetto, B., Rascovan, N., Álvarez, R., Mentaberry, A., Vázquez, M.P., 2014. Structure, Composition and Metagenomic Profile of Soil Microbiomes Associated to Agricultural Land Use and Tillage Systems in Argentine Pampas. PLoS ONE 9, e99949. doi:10.1371/journal.pone.0099949

Cardenas, E., Tiedje, J.M., 2008. New tools for discovering and characterizing microbial diversity. Curr. Opin. Biotechnol. 19, 544–9. doi:10.1016/j.copbio.2008.10.010

Ceja-Navarro, J.A., Rivera-Orduña, F.N., Patiño-Zúñiga, L., Vila-Sanjurjo, A., Crossa, J., Govaerts, B., Dendooven, L., 2010. Phylogenetic and Multivariate Analyses To Determine the Effects of Different Tillage and Residue Management Practices on Soil Bacterial Communities. Appl. Environ. Microbiol. 76, 3685–3691. doi:10.1128/AEM.02726-09

Chau, J.F., Bagtzoglou, A.C., Willig, M.R., 2011. The effect of soil texture on richness and diversity of bacterial communities. Environ. Forensics 12, 333–341.

Chen, W., Zhang, C.K., Cheng, Y., Zhang, S., Zhao, H., 2013. A Comparison of Methods for Clustering 16S rRNA Sequences into OTUs. PLOS ONE 8, e70837. doi:10.1371/journal.pone.0070837

Constancias, F., Saby, N.P.A., Terrat, S., Dequiedt, S., Horrigue, W., Nowak, V., Guillemin, J.-P., Biju-Duval, L., Chemidlin Prévost-Bouré, N., Ranjard, L., 2015. Contrasting spatial patterns and ecological attributes of soil bacterial and archaeal taxa across a landscape. MicrobiologyOpen 4, 518–531. doi:10.1002/mbo3.256

D'Amore, R., Ijaz, U.Z., Schirmer, M., Kenny, J.G., Gregory, R., Darby, A.C., Shakya, M., Podar, M., Quince, C., Hall, N., 2016. A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. BMC Genomics 17. doi:10.1186/s12864-015-2194-9

Degrune, F., Dufrêne, M., Colinet, G., Massart, S., Taminiau, B., Bodson, B., Hiel, M.-P., Daube, G., Nezer, C., Vandenbol, M., 2015. A novel sub-phylum method discriminates better the impact of crop management on soil microbial community. Agron. Sustain. Dev. 1–10.

Degrune, F., Theodorakopoulos, N., Colinet, G., Hiel, M.-P., Bodson, B., Taminiau, B., Daube, G., Vandenbol, M., Hartmann, M., 2017. Dynamics of microbial communities over the growing season in two contrasted tillage regimes.

Degrune, F., Theodorakopoulos, N., Dufrêne, M., Colinet, G., Bodson, B., Hiel, M.-P., Taminiau, B., Nezer, C., Daube, G., Vandenbol, M., 2016. No favorable effect of reduced tillage on microbial community diversity in a silty loam soil (Belgium). Agric. Ecosyst. Environ. 224, 12–21.

Dorr de Quadros, P., Zhalnina, K., Davis-Richardson, A., Fagen, J.R., Drew, J., Bayer, C., Camargo, F.A., Triplett, E.W., 2012. The effect of tillage system and crop rotation on soil microbial diversity and composition in a subtropical Acrisol. Diversity 4, 375–395. doi:10.3390/d4040375

East, R., 2013. Microbiome: Soil science comes to life. Nature 501, S18–S19.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.

Eylenbosch, D., Fernández Pierna, J.A., Baeten, V., Bodson, B., 2015. Use of NIR hyperspectral imaging and chemometrics to quantify roots and crop residues in soil.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.

Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecol. Lett. 12, 1238–1249. doi:10.1111/j.1461-0248.2009.01360.x

Ghyselinck, J., Pfeiffer, S., Heylen, K., Sessitsch, A., De Vos, P., 2013. The effect of primer choice and short read sequences on the outcome of 16S rRNA gene based diversity studies. PLoS One 8, e71360.

Giller, K.E., Witter, E., Mcgrath, S.P., 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem. 30, 1389–1414.

Goldfarb, K.C., Karaoz, U., Hanson, C.A., Santee, C.A., Bradford, M.A., Treseder, K.K., Wallenstein, M.D., Brodie, E.L., 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Front. Microbiol. 2, 94.

Gonzalez, A., Knight, R., 2012. Advancing analytical algorithms and pipelines for billions of microbial sequences. Curr. Opin. Biotechnol. 23, 64–71. doi:10.1016/j.copbio.2011.11.028

Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M., Whiteley, A.S., 2011. The bacterial biogeography of British soils. Environ. Microbiol. 13, 1642–1654.

Hamza, M.A., Anderson, W.K., 2005. Soil compaction in cropping systems: A review of the nature, causes and possible solutions. Soil Tillage Res. 82, 121–145. doi:10.1016/j.still.2004.08.009

Hao, X., Jiang, R., Chen, T., 2011. Clustering 16S rRNA for OTU prediction: a method of unsupervised Bayesian clustering. Bioinformatics 27, 611–618. doi:10.1093/bioinformatics/btq725

Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J. 9, 1177–1194. doi:10.1038/ismej.2014.210

Hartmann, M., Niklaus, P.A., Zimmermann, S., Schmutz, S., Kremer, J., Abarenkov, K., Lüscher, P., Widmer, F., Frey, B., 2014. Resistance and resilience of the forest soil microbiome to logging-associated compaction. ISME J. 8, 226–244.

Hartmann, M., Widmer, F., 2006. Community Structure Analyses Are More Sensitive to Differences in Soil Bacterial Communities than Anonymous Diversity Indices. Appl. Environ. Microbiol. 72, 7804–7812. doi:10.1128/AEM.01464-06

Hillel, D., 1998. Environmental soil physics: Fundamentals, applications, and environmental considerations. Academic press.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., Wardle, D.A., 2005. Effects of

Biodiversity on Ecosystem Functioning: A Consensus of Current Knowledge. Ecol. Monogr. 75, 3–35. doi:10.1890/04-0922

Hug, L.A., Baker, B.J., Anantharaman, K., Brown, C.T., Probst, A.J., Castelle, C.J., Butterfield, C.N., Hernsdorf, A.W., Amano, Y., Ise, K., Suzuki, Y., Dudek, N., Relman, D.A., Finstad, K.M., Amundson, R., Thomas, B.C., Banfield, J.F., 2016. A new view of the tree of life. Nat. Microbiol. 1, 16048. doi:10.1038/nmicrobiol.2016.48

Hughes, J.B., Hellmann, J.J., Ricketts, T.H., Bohannan, B.J.M., 2001. Counting the Uncountable: Statistical Approaches to Estimating Microbial Diversity. Appl. Environ. Microbiol. 67, 4399–4406. doi:10.1128/AEM.67.10.4399-4406.2001

Jiménez-Bueno, N.G., Valenzuela-Encinas, C., Marsch, R., Ortiz-Gutiérrez, D., Verhulst, N., Govaerts, B., Dendooven, L., Navarro-Noya, Y.E., 2016. Bacterial indicator taxa in soils under different long-term agricultural management. J. Appl. Microbiol. n/a-n/a. doi:10.1111/jam.13072

Krause, S., Le Roux, X., Niklaus, P.A., Van Bodegom, P.M., Lennon, J.T., Bertilsson, S., Grossart, H.-P., Philippot, L., Bodelier, P.L.E., 2014. Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Aquat. Microbiol. 5, 251. doi:10.3389/fmicb.2014.00251

Labreuche, J., Laurent, F., Roger-Estrade, J., 2014. Faut-il travailler le sol ? Acquis et innovations pour une agriculture durable, Quae, Arvalis Institut du végétal. ed, Savoir faire. Quae, Paris, France.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. Appl. Environ. Microbiol. 75, 5111–5120. doi:10.1128/AEM.00335-09

Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., Pierre, K.L., Risch, A.C., Seabloom, E.W., Schütz, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proc. Natl. Acad. Sci. 112, 10967–10972. doi:10.1073/pnas.1508382112

Lüdemann, H., Arth, I., Liesack, W., 2000. Spatial Changes in the Bacterial Community Structure along a Vertical Oxygen Gradient in Flooded Paddy Soil Cores. Appl. Environ. Microbiol. 66, 754–762. doi:10.1128/AEM.66.2.754-762.2000

Martiny, A.C., Treseder, K., Pusch, G., 2013. Phylogenetic conservatism of functional traits in microorganisms. ISME J. 7, 830–838.

Martiny, J.B.H., Jones, S.E., Lennon, J.T., Martiny, A.C., 2015. Microbiomes in light of traits: A phylogenetic perspective. Science 350, aac9323-aac9323. doi:10.1126/science.aac9323

McGill, B.J., Enquist, B.J., Weiher, E., Westoby, M., 2006. Rebuilding community ecology from functional traits. Trends Ecol. Evol. 21, 178–185.

Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670.

Navarro-Noya, Y.E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M.C., Valenzuela-Encinas, C., Jiménez-Bueno, N., Verhulst, N., Govaerts, B., Dendooven, L., 2013a. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biol. Biochem. 65, 86–95. doi:10.1016/j.soilbio.2013.05.009

Navarro-Noya, Y.E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M.C., Valenzuela-Encinas, C., Jiménez-Bueno, N., Verhulst, N., Govaerts, B., Dendooven, L., 2013b. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biol. Biochem. 65, 86–95. doi:10.1016/j.soilbio.2013.05.009

Nelson, W.C., Stegen, J.C., 2015. The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle. Front. Microbiol. 6. doi:10.3389/fmicb.2015.00713

Philippot, L., Andersson, S.G., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., Hallin, S., 2010. The ecological coherence of high bacterial taxonomic ranks. Nat. Rev. Microbiol. 8, 523–529.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., 2013. Going back to the roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789–799. doi:10.1038/nrmicro3109

Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., Lind, E.M., Seabloom, E.W., Adler, P.B., Bakker, J.D., others, 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecol. Lett. 18, 85–95.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. doi:10.1093/nar/gks1219

Quince, C., Lanzén, A., Curtis, T., Davenport, R., Hall, N., Head, I., Read, L., Sloan, W., 2009. Accurate determination of microbial diversity from 454 pyrosequencing data. Nat. Methods 6, 639–641. doi:10.1038/nmeth.1361

Quince, C., Lanzen, A., Davenport, R.J., Turnbaugh, P.J., 2011. Removing Noise From Pyrosequenced Amplicons. BMC Bioinformatics 12, 38. doi:10.1186/1471-2105-12-38

Ricketts, T.H., Watson, K.B., Koh, I., Ellis, A.M., Nicholson, C.C., Posner, S., Richardson, L.L., Sonter, L.J., 2016. Disaggregating the evidence linking biodiversity and ecosystem services. Nat. Commun. 7, 13106. doi:10.1038/ncomms13106

Roisin, C., 1997. Le semis sans labour en betterave sucriere. Etat actuel des connaissances. Cent. Rech. Agron. Gembloux, Nouvelles alternatives en matière de travail du sol: conséquences agronomiques et critères de choix.

Schimel, J., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Terr. Microbiol. 3, 348. doi:10.3389/fmicb.2012.00348

Sengupta, A., Dick, W.A., 2015. Bacterial Community Diversity in Soil Under two Tillage Practices as Determined by Pyrosequencing. Microb. Ecol. 70, 853–859. doi:10.1007/s00248-015-0609-4

Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32, 2099–2103.

Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Sci. Soc. Am. J. 63, 1350–1358.

Soane, B.D., Ball, B.C., Arvidsson, J., Basch, G., Moreno, F., Roger-Estrade, J., 2012. No-till in northern, western and south-western Europe: A review of problems and opportunities for crop production and the environment. Soil Tillage Res. 118, 66–87.

Soane, B.D., van Ouwerkerk, C., 2013. Soil compaction in crop production. Elsevier.

Tilman, D., 1982. Resource Competition and Community Structure. Princeton University Press Princeton, NJ.

Trognitz, F., Hackl, E., Widhalm, S., Sessitsch, A., 2016. The role of plant–microbiome interactions in weed establishment and control. FEMS Microbiol. Ecol. 92, fiw138.

Tullii, J., 2016. Etude de l'influence des pratiques culturales de conservation sur la dynamique des matières oganiques dans des contextes pédologiques contrastés (Travail de fin d'études). Université de Liège, Gembloux.

Tuomisto, H., 2012. An updated consumer's guide to evenness and related indices. Oikos 121, 1203–1218. doi:10.1111/j.1600-0706.2011.19897.x

Turmel, M.-S., Speratti, A., Baudron, F., Verhulst, N., Govaerts, B., 2014. Crop residue management and soil health: A systems analysis. Agric. Syst. doi:10.1016/j.agsy.2014.05.009

Ulrich, A., Becker, R., 2006. Soil parent material is a key determinant of the bacterial community structure in arable soils. FEMS Microbiol. Ecol. 56, 430–443.

van der Heijden, M.G. a, Bardgett, R.D., van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11, 296–310. doi:10.1111/j.1461-0248.2007.01139.x

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., Garnier, E., 2007. Let the concept of trait be functional! Oikos 116, 882–892. doi:10.1111/j.2007.0030-1299.15559.x

Wieder, W.R., Grandy, A.S., Kallenbach, C.M., Taylor, P.G., Bonan, G.B., 2015. Representing life in the Earth system with soil microbial functional traits in the MIMICS model. Geosci. Model Dev. 8, 1789–1808. doi:10.5194/gmd-8-1789-2015

Yeoh, Y.K., Sekiguchi, Y., Parks, D.H., Hugenholtz, P., 2015. Comparative Genomics of Candidate Phylum TM6 Suggests That Parasitism Is Widespread and Ancestral in This Lineage. Mol. Biol. Evol. msv281. doi:10.1093/molbev/msv281

Zhang, T., Shao, M.-F., Ye, L., 2012. 454 Pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. ISME J. 6, 1137–1147.