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Applications of C and N stable isotopes to ecological and environmental studies in seagrass ecosystems

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Abstract

Stable isotopes of carbon and nitrogen are increasingly used in marine ecosystems, for ecological and environmental studies. Here, we examine some applications of stable isotopes as ecological integrators or tracers in seagrass ecosystem studies. We focus on both the use of natural isotope abundance as food web integrators or environmental tracers and on the use of stable isotopes as experimental tools. As ecosystem integrators, stable isotopes have helped to elucidate the general structure of trophic webs in temperate, Mediterranean and tropical seagrass ecosystems. As environmental tracers, stable isotopes have proven their utility in sewage impact measuring and mapping. However, to make such environmental studies more comprehensible, future works on understanding of basic reasons for variations of N and C stable isotopes in seagrasses should be encouraged. At least, as experimental tracers, stable isotopes allow the study of many aspects of N and C cycles at the scale of a plant or at the scale of the seagrass ecosystem.

Keywords: Sewage; Food web; Stable isotopes; Seagrass; Tracer

1. Introduction

Stable isotopes are used as integrators and tracers of ecological processes at both naturally occurring levels and experimentally enriched abundances (i.e. at levels outside the natural range of values due to the addition of labelled substances) (Robinson, 2001). They provide ecological information across a range of spatio-temporal scales, i.e. from cell to ecosystems, and across a time scale of seconds to millennia (Dawson et al., 2002).

Here, we illustrate these different applications to demonstrate that stable isotopes (mainly C and N) are essential tools in the study of all aspects of seagrass ecosystem functioning. We focus on environmental applications of stable isotopes in seagrass ecosystems.

2. Natural abundance of C and N stable isotopes in seagrasses

2.1. Natural variations

Natural isotopic abundance is reported on a delta scale (δ) which indicates the deviation (in ‰) of the isotopic composition of a sample from an internationally accepted standard (e.g. Robinson, 2001).

Delta values of nitrogen stable isotopes ($\delta^{15}N$) in seagrasses vary from -2‰ (Mac Clelland et al., 1997) to 12.3‰ (Fourqurean et al., 1997), but the most frequent values are lie between 0‰ and 8‰, depending on the seagrass ecosystem (e.g. Anderson and Fourqurean, 2003; Lepoint et al, 2003; Marguillier et al., 1998; Melville and Connolly, 2003; Vizzini and Mazzola, 2003). The $\delta^{15}N$ variations are not well understood but are related to inorganic N incorporation by seagrasses and to sediment and column water geochemistry (e.g. Fourqurean et al., 1997). $\delta\ ^{15}N$ values close to 0‰ are often attributed to N_2 fixation by associated seagrass organisms (e.g. Yamamuro et al., 2003).

Delta values of carbon ($\delta^{13}C$) in seagrasses range from — 23‰ to — 3‰, but the most frequent values are around — 10‰ (Hemminga and Mateo, 1996). These values are high compared to other marine primary producers, although macro-algae can also have values as high as this (Raven et al., 1995). Primarily, the plant's $\delta^{13}C$ values are determined during photosynthesis. The high $\delta^{13}C$ values of seagrasses are partly related to their ability to use bicarbonate as an inorganic carbon source (e.g. Beer et al., 2002). Indeed, bicarbonate has a less negative $\delta^{13}C$ than CO_2 (0‰ vs. -9‰) and its incorporation by the plant may lead to a relatively high $\delta^{13}C$ (Raven et al., 2002). In addition, variations of photosynthesis rate and irradiance level induce variations of the isotopic discrimination (i.e. extent of changes in partitioning of ^{13}C and ^{12}C between the inorganic source and organic product) (e.g. Grice et al., 1996; Hemminga and Mateo, 1996). As photosynthesis rate and irradiance level vary both temporally and spatially, the $\delta^{13}C$ of seagrasses is often depth-related and shows variations according to season, location and community structure (e.g. Anderson and Fourqurean, 2003; Boyce et al., 2001; Lepoint et al., 2003; Rose and Dawes, 1999; Viz-zini et al., 2003).

2.2. Food web integrators

$\delta\ ^{13}C$ may sometimes define an isotopic signature for seagrass, distinguishable from those of other primary producers. For example, in a Corsican seagrass bed, it is possible to assign an isotopic signature to the phyto-plankton (-23‰), to dominant macroalgae (-19‰) and to seagrass leaves (-9‰) (Dauby, 1989). These signatures of potential food sources, and the fact that the isotopic composition of an animal is strongly determined by the isotopic composition of its food, allow the use of isotopic ratios as food web integrators. On the other hand, $\delta\ ^{15}N$ offers the possibility of estimating the trophic level of organisms, because $\delta\ ^{15}N$ values generally increase with increasing trophic position (e.g. Hobson and Welsh, 1992) but this ^{15}N enrichment is variable; it varies between animal groups and is often diet-related (e.g. Mac Cutchan et al., 2003; Vanderklift and Ponsard, 2003). Stable isotopes (C, N, S) have been largely used to assess seagrass food webs in temperate meadows (e.g. Kharlamenko et al., 2001; Stephenson et al., 1986), in Mediterranean meadows (e.g. Dauby, 1989; Jennings et al., 1997; Lepoint et al., 2000; Pinnegar and Polunin, 2000; Vizzini and Mazzola, 2003), and in subtropical and tropical meadows (e.g. Fry, 1984; Kitting et al., 1984; Loneragan et al., 1997; Marguillier et al., 1998; Melville and Connolly, 2003; Moncrieff and Sullivan, 2001).

2.3. Environmental tracers

Seagrasses are very sensitive to water quality changes induced by human activities, particularly to nutrient load increase due to sewage effluent or mariculture activities (e.g. Cloern, 2001; Holmer et al., 2003). Stable isotopes analysis of plant material offers the possibility of detecting the biological role of ground water flow in the marine environment (Kamermans et al., 2002) or the impact of sewage effluent before major ecological changes occur (Mac Clelland et al., 1997; Mac Clelland and Valiela, 1998). It is particularly useful in areas where a small nutrient increase could have a significant impact on the ecosystem especially where this nutrient increase is undetectable in the water due to, for example, a low sewage load or rapid dilution in the surrounding environment (e.g. in coral reef waters, oligotrophic coastal areas and seagrass ecosystems) (Gartner et al., 2002; Yamamuro et al., 2003).

Stable isotope use in tracing waste or ground water in the marine environment is primarily based on the possibility of distinguishing the different N inorganic sources by their isotopic signatures. For example, in the Waquoit Bay (Massachusetts, USA), isotopic studies have permitted the attribution of an isotopic signature to nitrates from waste water, from fertiliser and from atmospheric deposition (Mac Clelland et al., 1997). The ^{15}N isotopic composition of primary producers partly reflects the isotopic composition of their N sources. Nitrogen from waste water has generally higher $\delta\ ^{15}N$ than inorganic nitrogen from marine environment, because of their human or animal origin (i.e. high trophic level origin) and because of isotopic discrimination during re-mineralisation processes (i.e. volatilisation of ^{14}N-ammonium during ammonification) (Macko and Ostrom, 1994).

However, high values in seagrass material are not necessarily the reflection of sewage or ground water impacts. For example, Fourqurean et al. (1997) measured the increase of $\delta\ ^{15}N$ values of *Zoster a marina* from the mouth to the head of Tomales Bay in California where these values become very high (+12‰). In this relatively preserved bay, the ground water discharges are considered low. The high $\delta\ ^{15}N$ values are attributed to the occurrence of deni-trification processes in Tomales Bay marine waters, which may have resulted in the ^{15}N enrichment of the remaining inorganic N pool (e.g. Horrigan et al., 1990), and, consequently, a ^{15}N enrichment of plants which incorporate inorganic N from the water column.

Isotopic measurements in plants can be done more routinely than in water or sediment, allowing the collection and measurement of a high number of samples, which is necessary in the mapping or measuring of sea-wage impacts (Costanzo et al., 2001). Plankton is generally not considered to be a good tracer for isotopic environmental studies because of its short turnover time and the variability of its isotopic signature, which is often independent of the variability of the $\delta\ ^{15}N$ of sources, (Cabana and Rasmussen, 1996). On the contrary, use of benthic plants allows a temporal integration of the ^{15}N source signal because of their longer turnover time

(Costanzo et al., 2001). However, sea-grasses often display very complex strategies to meet their nutrient requirements, particularly of nitrogen (Touchette and Burkholder, 2000). Some species or populations rely almost exclusively on nutrients of the water column, others on the nutrients of the pore water pool, while a majority of species rely on a variable mixture of these two sources. In these conditions, seagrasses should be poorer indicators of water column process than, for example, macroalgae which rely only on their surrounding water for their nutrient requirements (e.g. Gartner et al., 2002; Umezawa et al., 2002). On the other hand, the $\delta^{15}N$ of species or populations which uptake a significant part of their inorganic N from sediment pore water, should be an informative indicator of environmental conditions in the sediment.

3. Tracer experiments

Stable isotopes can be used as experimental tracers. Many "labelled" substances (i.e. substances with a proportion of one stable isotope, generally the heavier, clearly outside the range of its natural abundance in the unlabelled substance) exist now on the market. Experimental tracers allow us to study, and sometimes to quantify, in situ processes involving C and N at the scale of the plant or the community. At the plant or ramet level, these techniques have been use to increase the discrimination of isotopic signatures of primary producers for food web studies (Winning et al., 1999; Mutchler et al., 2004), to quantify N uptake rate and allocation (Iizumi and Hattori, 1982; Lepoint et al., 2002b, 2004b; Pedersen and Borum, 1992; Pedersen et al., 1997) or epiphytes (e.g. Cornelisen and Thomas, 2002), to assess N internal recycling (Borum et al., 1989; Lepoint et al., 2002a), to measure primary production (Mateo et al., 2001) and to assess N and C transfer between shoots (Libes and Boudouresque, 1987; Marba et al., 2002). At seagrass community level, ^{15}N tracer experiments have been performed to study the role of benthic vegetation as sinks of nitrogen inputs (e.g. Dudley et al., 2001; Lepoint et al., 2004a) or the retention efficiency of N in tropical seagrass ecosystems (e.g. Sta-pel et al., 2001).

4. Future prospect and perspectives

The basic reasons for the $\delta^{13}C$ and $\delta^{15}N$ values and the causes of their variations in seagrasses are not well known, particularly for $\delta^{15}N$ values. Specific experiments, like those done for terrestrial plants, should be encouraged. Secondly, recent advances in experimental tracer studies, offer the possibility to study the fundamental ecological process involved in C and N cycles, particularly at the community scale (e.g. Stapel et al., 2001). Thirdly, new tools such as the combination of bacterial biomarkers and stable isotopes (both natural and enriched abundance) open a horizon for the understanding of C and N organic matter fluxes in seagrass ecosystems (e.g. Boschker et al., 2000; Boschker and Middelburg, 2002).

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