Biol Fertil Soils (2010) 46:877–881 DOI 10.1007/s00374-010-0483-7

SHORT COMMUNICATION

Seasonal variation in nitrogen isotopic composition of bog plant litter during 3 years of field decomposition

Luca Bragazza · Paola Iacumin · Chiara Siffi · Renato Gerdol

Received: 2 December 2009 / Revised: 2 July 2010 / Accepted: 2 July 2010 / Published online: 17 July 2010 © Springer-Verlag 2010

Abstract In this study, we describe the seasonal variation in ¹⁵N abundance in the litter of two *Sphagnum* species and four vascular plant species during 3 years of field decomposition in an Italian Alpine bog. Litter bags were periodically retrieved at the end of summer and winter periods, and the $\delta^{15}N$ in residual litter was related to mass loss, litter chemistry, and climatic conditions. In Sphagnum litter, higher rates of decomposition during summer months were associated with an increase of $\delta^{15}N$ probably due to the incorporation of microbial organic compounds rich in ¹⁵N. The litter of *Eriophorum vaginatum* and *Carex* rostrata was characterized by a decrease of δ^{15} N, so that the final signature was significantly lower than in initial litter. On the other hand, the residual litter of Potentilla erecta and Calluna vulgaris was characterized by a final δ¹⁵N higher than in initial litter. Our data reported a seasonality of ¹⁵N abundance in the residual litter of *Sphagnum* species, but not in that of vascular plant species, thus highlighting the role of differences in litter chemistry.

Keywords δ^{15} N · Decomposition · Italian Alps · Litter C/N quotient · Peatland · *Sphagnum* · Vascular plants

Introduction

On a global scale, the natural abundance of nitrogen (N) stable isotope ratio (δ^{15} N) in leaves and bulk soil is known to increase with increasing mean annual temperature and to decrease with increasing mean annual precipitation (Amundson et al. 2003; Craine et al. 2009). In addition, Craine et al. (2009) have shown that mycorrhizal plant species have a lower foliar 15 N abundance than nomycorrhizal species and the largest depletion was observed for ericoid mycorrhizal symbiosis. Consequently, on a local scale, the dependence of foliar δ^{15} N on mycorrhizal association can significantly affect the 15 N abundance of initial plant litter accumulating on soil.

Many processes can alter the 15 N/ 14 N ratio of the source and the sink pool, but usually 15 N abundance increases with soil depth (Hogberg 1997). Among the N processes affecting δ^{15} N there are (1) ammonia volatilization, which discriminates against 15 N and enriches residual soil N in 15 N; (2) nitrification from NH₄ $^+$ to NO₃ $^-$ produces NO₃ $^-$ with a lower δ^{15} N and thus enriches the residual NH₄ $^+$ in 15 N; (3) denitrification, which releases N₂ depleted in 15 N, enriches the remaining NO₃ $^-$ in 15 N; (4) mycorrhizal fungi are enriched in 15 N so that the host plants can receive 15 N-depleted N compounds; (5) the mixing of soil layers by biotic and abiotic processes can affect the 15 N/ 14 N ratio (Hobbie and Ouimette 2009).

L. Bragazza (☒) · C. Siffi · R. Gerdol Department of Biology and Evolution, University of Ferrara, Corso Ercole I d'Este 32,

44100 Ferrara, Italy

e-mail: luca.bragazza@unife.it

L. Bragazza

WSL Swiss Federal Institute for Forest, Snow and Landscape Research, Site Lausanne, station 2, CH-1015 Lausanne, Switzerland

L. Bragazza

Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratory of Ecological Systems–ECOS, station 2, CH-1015 Lausanne, Switzerland

P. Iacumin

Department of Earth Sciences, University of Parma, V.le G.P. Usberti 157/A, Campus Universitario, 43100 Parma, Italy



In peatlands, a general increase of ¹⁵N abundance with peat depth has also been reported (Kohzu et al. 2003; Asada et al. 2005a; Cortizas et al. 2007) and attributed to microbial alteration of organic matter more than to accumulation of recalcitrant compounds (Kramer et al. 2003). However, a better understanding of the changes in ¹⁵N abundance in peat requires to know the changes in isotopic composition of fresh plant litter during early stages of decomposition, i.e., before plant remnants turn into peat because litter decomposition is a crucial process in controlling the rate of peat accumulation in peatlands (Bragazza et al. 2009). For example, Asada et al. (2005b) reported an increase of ¹⁵N abundance in the litter of the peat moss Sphagnum fuscum after 2 years of field decomposition as consequence of the incorporation of ¹⁵N-enriched N from the surrounding. Such a result was in accordance with Connin et al. (2001) reporting an increase of $\delta^{15}N$ in the litter of desert plant species over a 5-year period, but in contrast with Melillo et al. (1989) showing a decrease of $\delta^{15}N$ in pine needles over a 6-year period. Additional studies are then needed to better understand the extent of isotopic changes during shortterm litter decomposition in peatlands so as to allow the use of stable isotope ratios as a tracer of organic matter alteration and the origin of decomposed material.

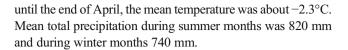
This paper is part of a broad study investigating the stable isotope composition (13 C and 15 N) in plant litter during field decomposition in an Alpine bog (Bragazza and Iacumin 2009). Here, we will describe the seasonal variation of 15 N abundance in the litter of two *Sphagnum* species and four vascular plant species during 3 years of decomposition. We used litter bags and periodically monitored the 15 N abundance of residual litter so as to relate the isotopic signature to mass loss, litter chemistry, and climatic conditions. We have hypothesized that seasonal microbial activity is primarily responsible for the changes of δ^{15} N in the residual plant litter.

Materials and methods

Study site

The study site (Marcesina peatland) is located on the Italian pre-Alps (45°57′ N; 11°37′ E), province of Vicenza, at an altitude of 1,300 ma.s.l. The Marcesina peatland has an area of about 2 ha and is prevalently fed by rain water.

Climatic data were collected from a meteorological station (Rifugio Marcesina) about 1 km away from the study bog. During the study period 2004–2007, the mean temperature of summer months, i.e., from the beginning of May until the end of September, was about 11.0°C, whereas during winter months, i.e., from the beginning of October



Litter bag preparation and sampling interval

The same dominant plant species previously selected for ¹³C monitoring were considered in the present study, that is, *Eriophorum vaginatum* L., *Carex rostrata* Stokes, *Calluna vulgaris* (L.) Hull, *Potentilla erecta* (L.) Raüschel, *Sphagnum fuscum* (Schimp.) Klinggr., and *Sphagnum magellanicum* Brid. (Bragazza and Iacumin 2009).

Litter bags were prepared according to Bragazza and Iacumin (2009). Very briefly, in mid-September 2004, freshly senescent and undecomposed leaves were collected from vascular plant species, whereas the stem segment between 2 and 4 cm below the growing tip was used as representative of *Sphagnum* litter. Litter bags were made of polyethylene fabric with 0.5-mm mesh size and contained about 1 g of air-dry *Sphagnum* litter or about 1.5 g of air-dry vascular plant litter. For each plant species, three subsamples of litter were oven dried for 48 h at 40°C to calculate oven-dry weight of each litter bag before burial.

At the beginning of October 2004, 48 litter bags for each plant species were buried into the peat soil at about 5-cm depth, in correspondence of the respective species habitats. Eight litter bags for each plant species were periodically sampled at the beginning of May and at the beginning of October. For simplicity, hereafter, we will call "winter period" the period from the beginning of October until the end of April and "summer period" the period from the beginning of May until the end of September. After sampling, each litter bag was cleaned from debris, dried at 40°C for 48 h, and then weighted.

Mass loss, isotopic, and chemical analyses

The percentage mass loss at time *t* and the seasonal rate of mass loss, i.e., the percentage mass loss during each single winter and summer period, were calculated according to Bragazza and Iacumin (2009). The rate of mass loss for each plant species has been reported by Bragazza and Iacumin (2009).

Total N and carbon (C) concentration and ^{15}N abundance were quantified in the residual litter with an elemental analyser (EA 1110, Carlo Erba, Milan, Italy) coupled online with an isotope ratio mass spectrometer (delta Plus XP, ThermoFinnigan, Bremen, Germany). In particular, N isotopic abundance is expressed as $\delta^{15}N$ and was calculated as follows:

$$\delta(\%_0) = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000$$



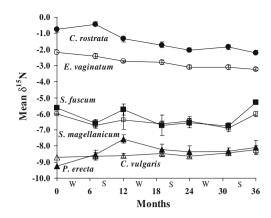


Fig. 1 Mean seasonal value (\pm SD) of δ^{15} N in *Sphagnum* litter and vascular plant litter during 3 years of field decomposition. The isotopic signature at time zero corresponds to the δ^{15} N of the initial litter. W is the winter period (from 1st October until the end of April), S is the summer period (from 1st May until the end of September)

where R is the 15 N/ 14 N ratio in the sample and the standard, being the latter atmospheric N₂. The standard deviation of replicates was better than 0.2‰.

All chemical analyses were performed after grinding and sieving (<0.1 mm) the litter samples. All concentrations were converted to standard oven-dry weight (40°C for 48h).

The seasonal change of mean $\delta^{15}N$ was calculated as difference between the mean value at time t and that at time t-1, i.e., $\delta^{15}N_t-\delta^{15}N_{t-1}$. The C/N quotient (weight/weight) was calculated for each litter bag basing on C and N concentration.

Statistical analysis

The effect of litter type (i.e., plant species) and time of burial on seasonal changes in $\delta^{15}N$ were tested by the two-way ANOVA, whereas the one-way ANOVA was applied to assess significant differences in litter chemistry within and among plant species. All statistical analyses were performed using Statistica for Windows v. 6.0.

Results and discussion

The δ^{15} N in initial litter differed among the selected plant species in the following sequence: Carex rostrata>E. vaginatum > S. fuscum = S. magellanicum > Calluna vulgaris>P. erecta (Fig. 1). These differences can be explained taking into account the presence and the type of mycorrhizal symbiosis. Indeed, the foliar $\delta^{15}N$ in ericoid mycorrhizal and ectomycorrhizal plants is typically lower than in arbuscular mycorrhizal and nonmycorrhizal plants (Michelsen et al. 1998; Craine et al. 2009) as a consequence of a greater discrimination against ¹⁵N by ericoid mycorrhizal and ectomycorrhizal fungi (Emmerton et al. 2001). Accordingly, the low δ^{15} N in *Calluna vulgaris* litter reflects the presence of ericoid mycorrhizal infection, which is known to express the greatest ¹⁵N discrimination, so that the fungal mycelium is enriched and the host plant is depleted in ¹⁵N (Michelsen et al. 1998; Nordbakken et al. 2003: Aerts et al. 2009). On the other hand, the higher δ^{15} N in Carex rostrata and E. vaginatum litter is in accordance with the absence of mycorrhizal infection in these two Cyperaceae (Miller 1982; Thormann et al. 1999; Asada et al. 2005a). Rather surprisingly, P. erecta litter had the lowest ¹⁵N abundance, although this species is characterized by arbuscular mycorrhizae (Harley and Harley 1987; Titus and Leps 2000). Anyway, Craine et al. (2009) reported a great variation of foliar $\delta^{15}N$ in arbuscular mycorrhizal plants suggesting the transfer of ¹⁵N-depleted N from the mycorrhizal fungi to the host plant in a similar way to ectomycorrhizal plants (Leigh et al. 2009). However, the extent to which the arbuscular mycorrhizal symbiosis can affect the foliar $\delta^{15}N$ of host plant is still rather unclear (Hobbie and Hobbie 2008).

The $\delta^{15}N$ of initial *Sphagnum* litter did not differ significantly between *S. fuscum* and *S. magellanicum* and was comparable to previous values reported for ombrotrophic *Sphagnum* species of the Italian Alps (Bragazza et al. 2005). Because peat mosses typically rely on atmospheric deposition as primary source of nutrients (Nordbakken et al.

Table 1 The C/N quotient in initial litter and during the decomposition period

	Mean initial C/N quotient	Average C/N quotient during 3years	Correlation value C/N vs. δ ¹⁵ N
S. fuscum	74.5 (2.9)	71.8 (6.7)	n.s.
S. magellanicum	68.0 (3.0)	61.3 (5.0)	n.s.
Carex rostrata	42.7 (4.7)	29.6 (4.5)	0.87 (<i>P</i> <0.01)
E. vaginatum	38.5 (1.5)	21.8 (3.7)	0.88 (<i>P</i> <0.01)
Calluna vulgaris	49.2 (2.3)	30.8 (3.7)	n.s.
P. erecta	48.1 (1.0)	32.8 (3.1)	n.s.

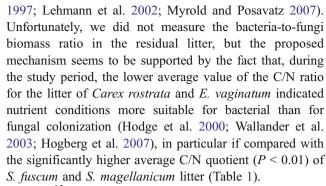
The correlation value between mean C/N quotient and corresponding mean δ^{15} N during 3 years of field decomposition is also reported. Values are the average (\pm SD) of five replicates for initial litter and six seasonal mean values for the average C/N quotient. Correlation is based on Pearson's *R* value *n.s.* not significant



2003), the ¹⁵N abundance in initial *Sphagnum* litter seems then to reflect the N isotopic signature of local atmospheric deposition (Bragazza et al. 2005; Zechmeister et al. 2008).

According to the two-way ANOVA, the $\delta^{15}N$ of residual litter differed significantly among plant species and sampling periods (P<0.01) with a significant interaction between plant species and time of sampling (P<0.01). In the case of *Sphagnum* litter, mean $\delta^{15}N$ in initial and final litter did not differ significantly, but we observed that the average value of δ^{15} N in S. fuscum and in S. magellanicum litter at the end of summer periods $(-5.90\pm0.66 \text{ and } -6.30\pm$ 0.44, respectively) was significantly higher (P < 0.01; n = 24per species) than at the end of winter periods (-6.69 ± 0.42) and -6.75 ± 0.35 , respectively), thus indicating an enrichment in 15N of residual litter by the increase of air temperature (Fig. 1). In addition, the mean litter C/N quotient was negatively correlated with the mean percentage of mass loss (Pearson's R < -0.53, P < 0.05; n = 6 per species). We hypothesize that the relative increase of $\delta^{15}N$ during summer months could reflect a higher microbial growth and activity (Gioacchini et al. 2006), in particular the incorporation of microbial organic compounds by Sphagnum residual litter (Connin et al. 2001; Kramer et al. 2003; Asada et al. 2005a; Huygens et al. 2008). This hypothesis is supported by the fact that microbial biomass is generally enriched in ¹⁵N compared to the bulk soil (Dijkstra et al. 2006; Coyle et al. 2009). A similar mechanism has been proposed for explaining the summer enrichment of ¹³C abundance in the same litter bags (Bragazza and Iacumin 2009). Indeed, in Sphagnum litter, the seasonal change of mean δ^{15} N was positively correlated with the corresponding seasonal change of mean δ^{13} C (Pearson's R=0.63, P=0.051;

In vascular plant litter, the average value of $\delta^{15}N$ at the end of summer periods and that at the end of winter periods did not differ significantly, thus indicating the absence of seasonal differences in the 15N abundance. The litter of Carex rostrata and E. vaginatum was characterized by a decrease of $\delta^{15}N$ so that the final ^{15}N abundance was significantly lower (P < 0.01) than both values of the initial litter (Fig. 1). In addition, the mean litter C/N quotient was positively correlated with the corresponding mean $\delta^{15}N$ (Table 1) indicating that with increasing N over C retention, the residual litter was more and more depleted in ¹⁵N. We hypothesize that the decrease of 15N abundance could reflect the taxonomical diversity of decomposing microbes. Indeed, the dominance of fungi in decomposing litter is supposed to enrich the litter with microbial products having a higher δ^{15} N, being fungal mycelium typically enriched in ¹⁵N (Hobbie and Colpaert 2003; Lindahl et al. 2007; Mayor et al. 2009). Instead, a dominance of bacteria can decrease the $\delta^{15}N$ of residual litter because bacteria have a greater potential for immobilizing nitrate depleted in ¹⁵N (Hogberg



The $\delta^{15}N$ of *P. erecta* litter showed a gradual increase during the first year of burial with a corresponding mean mass loss of 84% $\pm 4.2\%$, then the δ^{15} N decreased to reach a mean final value that was significantly higher (P < 0.05) than that of the initial litter (Fig. 1). In addition, there was a significant correlation between the mean percentage of mass loss and the corresponding mean $\delta^{15}N$ (Pearson's R=0.83, P < 0.05; n = 7, including initial value), thus supporting the hypothesis that the increase in the ¹⁵N of *P. erecta* litter could be due to microbial activity (Kramer et al. 2003; Asada et al. 2005b). A similar mechanism could also be proposed for Calluna vulgaris litter considering the positive correlation between mean percentage of mass loss and mean $\delta^{15}N$ (Pearson's R=0.75, P=0.051; n=7 including initial value), although the final ¹⁵N abundance did not differ significantly from initial value (Fig. 1). The smaller N isotopic changes in Calluna vulgaris litter compared to P. erecta litter were in accordance with the significantly different decomposition rate after 3 years, i.e., 69.8% ±8.1% for Calluna vulgaris and 91.8%±1.6% for *P. erecta*. It is worthy to note that the average C/N quotient for P. erecta and Calluna vulgaris litter was significantly higher (P<0.01) than that of Carex rostrata and E. vaginatum litter (Table 1), thus supporting the hypothesis that decomposition was primarily driven by fungi in Calluna vulgaris and P. erecta litter.

Acknowledgements Thanks are due to I. Targa for field assistance and to R. Marchesini and F. Tressino for laboratory assistance. R. Gerdol acknowledges the funding of this study by the "Fondazione Cassa di Risparmio di Verona, Vicenza, Belluno e Ancona" through the project "R.I.T.A.", whereas L. Bragazza acknowledges the contribution by the useful discussions during the NSF-RCN (U.S.)-sponsored PEATNET Workshop "Why is There Peat?" (Villanova University, March 27–28, 2008). Helpful comments and suggestions from P. Nannipieri and three anonymous reviewers are also acknowledged.

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