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Original article

^{13}C and ^{15}N isotopic fractionation in trees, soils and fungi in a natural forest stand and a Norway spruce plantation*

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Abstract – ^{15}N and ^{13}C natural abundances of foliage, branches, trunks, litter, soil, fungal sporophores, mycorrhizas and mycelium were determined in two forest stands, a natural forest and a Norway spruce plantation, to obtain some insights into the role of the functional diversity of saprotrophic and ectomycorrhizal fungi in carbon and nitrogen cycles. Almost all saprotrophic fungi sporophores were enriched in ^{13}C relative to their substrate. In contrast, they exhibited no or very little shift of $\delta^{15}N$. Judging from the amount of C discrimination, ectomycorrhizal fungi seem to acquire carbon from their host or from dead organic matter. Some ectomycorrhizal species seem able to acquire nitrogen from dead organic matter and could be able to transfer it to their host without nitrogen fractionation, while others supply their host with ^{15}N-depleted nitrogen. Moreover ectomycorrhizal species displayed a significant N fractionation during sporophore differentiation, while saprotrophic fungi did not.

^{13}C / ^{15}N / forest stands / saprotrophic fungi / ectomycorrhizal fungi

Résumé – Fractionnement isotopique 13C et 15N dans les arbres, le sol et les champignons pour un peuplement de forêt naturelle et une plantation d'épicéas. Les abondances naturelles du ^{15}N et du ^{13}C de la masse foliaire, des branches, des troncs, de la litière, du sol, des carpophores, des mycorhizes et du mycélium, ont été déterminées dans deux peuplements forestiers, une forêt naturelle et une plantation d'épicéas, afin d'obtenir quelques précisions sur le rôle de la diversité fonctionnelle des champignons saprophytes et ectomycorhiziens dans le cycle du carbone et de l'azote. Presque tous les champignons saprophytes présentent un enrichissement en ^{13}C relativement à leur substrat. Par contre, ils ne présentent pas ou ne présentent que très peu de modifications du $\delta^{15}N$. En fonction de leur taux de discrimination du carbone, les champignons ectomycorhiziens semblent pouvoir acquérir du carbone à la fois à partir de leur hôte et de la matière organique morte. Quelques espèces semblent capables d'acquérir de l'azote organique du sol et de le transférer sans fractionnement à leur hôte alors que d'autres fournissent leur hôte en azote appauvri en ^{15}N. De plus, les espèces ectomycorhiziennes présentent un fractionnement significatif de l'azote pendant la différenciation des carpophores, alors que les champignons saprophytes n'en présentent pas.

13C / 15N / peuplements forestiers / champignons saprophytes / champignons mycorhiziens

1. INTRODUCTION

In forest ecosystems, litter and wood breakdown is crucial for nutrient cycling, especially for nitrogen. Saprotrophic fungi (SF) play a central role in this cycling. They are the most important decomposers of organic matter, from which they gain their energy besides other important nutrients [52]. Ectomycorrhizal fungi (EMF) are essential to the health and growth of forest trees [54]. They can benefit forest trees in a number of ways, although the most important is the enhancement of nutrient absorption from soil [21]. For organic matter breakdown, nutrient cycling and energy remobilisation, the interactions between saprotrophic and ectomycorrhizal fungi are complex [45]. The general assumption that saprotrophic fungi would do the mineralization alone and that the ectomycorrhizal fungi would take up the mineral elements resulting from this process is a simplistic view. The 'Gadgil effect' is a good example of the interaction complexity between both fungal groups. In New Zealand, in field and laboratory conditions, when ectomycorrhizas were excluded from *Pinus radiata* litter, the rate of litter decomposition increased over a 12-month period [15, 16]. Several explanations have been proposed for the 'Gadgil effect'. It was attributed to stimulated colonization and exploitation of litter by EMF at the expense of litter SF due to direct inhibition of SF by EMF. Although ectomycorrhizal fungi are able to break down litter organic matter, exploitation of litter by EMF in preference to SF would therefore result in reduced rates of litter decomposition [7]. It was shown that litter moisture content was also reduced as ectomycorrhiza density increased [43]. Moisture content is a key determinant of forest litter decomposition, affecting the size, composition

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and activities of saprotrophic communities [53]. These interactions must be further complicated by the considerable functional diversity that exists between different species of EMF and SF [5]. Direct competition between ectomycorrhizal and saprotrophic fungi for nitrogen has also been implicated in the 'Gadgil effect'.

The importance of ectomycorrhizas for nitrogen nutrition was recognized at an early stage [50]. Mineral nitrogen levels in the soil solution represent a low percentage of nitrogen potentially available for uptake. It is likely that the uptake of nitrogen largely occurs through EM fungi, as their extraradical hyphae commonly make up most of the nutrient absorption surface of the tree [55]. Ammonium often is the dominant form of inorganic N in forest soils. Ectomycorrhizal fungi have a preference for NH_4^+ and there is considerable variability in their ability to utilize NO_3^- [49]. In the view of many authors, inorganic N absorbed into hyphae is assimilated and translocated as amides and amino acids in the fungus, probably utilizing metabolic pathways that are different from those of the host plant [6, 10, 14, 47, 48]. It has been shown that some ectomycorrhizas can produce proteolytic enzymes, which release and take up N from various peptides [1, 2] and this may contribute to direct cycling of N through forest floor litter [51]. It is clear that EMF have a potential to mineralize and also directly gain resources from complex organic soil fractions.

However, the quantitative significance of direct N or C cycling by ectomycorrhizal fungi in the field is not very well known. Stable isotope techniques are efficient tools for ecophysiology and ecosystem research [25, 35, 37]. ^{13}C and ^{15}N natural abundance and ^{14}C measurements have been used to study fungal sources of carbon and nitrogen [28]. Gebauer and Dietrich [17] found that sporophores of ectomycorrhizal fungi were more enriched in ^{15}N than other ecosystem components including sporophores of saprotrophic fungi. Högberg et al. [36] showed that ectomycorrhizas of Norway spruce and beech collected across Europe were 2‰ more enriched in ^{15}N than non-mycorrhizal fine roots. Fungal sheaths were 2.4–6.4‰ enriched relative to the root core. Other studies have confirmed that sporophores of ectomycorrhizal fungi were often enriched in ^{15}N relative to sporophores of saprophytic fungi, whereas sporophores of saprotrophic fungi were almost all the time enriched in ^{13}C relative to sporophores of ectomycorrhizal fungi [23, 26, 27, 41, 55–57]. Taylor et al. [56] shown that isotopes signatures of sporophores varied by family, genus and species. Lilleskov et al. [44] showed a correlation between isotope signatures and possible ecophysiological functions. Ectomycorrhizal fungal species that utilized organic nitrogen in laboratory cultures exhibited higher natural abundance of $\delta^{15}N$ than did fungal species that utilized only inorganic forms of nitrogen. Emmerton et al. [12] used only fungi to show that there was fractionation of N isotopes upon uptake. However, the concentration of the inorganic N compounds in the experiment was much higher than that found in nature. These laboratory results, therefore, cannot be extrapolated to the natural situation.

Although the relative contribution of the C and N sources and the different internal processes involved in the fractionation of ^{13}C and ^{15}N remain unclear, it appears that the analysis of natural abundances of carbon and nitrogen isotopes could provide an insight into the respective trophic role of saprotrophic versus ectomycorrhizal fungi [18, 26–28, 31–34, 37, 38].

The purpose of this work was to investigate the ways of nitrogen and carbon acquisition by both fungal types in a natural mixed forest stand and a Norway spruce plantation, situated in the centre of France, by using ^{13}C and ^{15}N natural abundance. The aims of this work were (i) to determine whether ^{13}C and ^{15}N natural abundance could differentiate the ecological groups of Basidiomycetes present at the two sites, (ii) to determine whether ectomycorrhizal fungi were able to acquire carbon from dead organic matter in addition to the carbon provided by their host, (iii) to determine the possible role of hosts in carbon and nitrogen acquisition by ectomycorrhizal fungi, (iv) to determine whether the processes involved in mycorrhizal functioning and sporophore differentiation could partly explain differences in N fractionation generally observed between ectomycorrhizal and saprotrophic fungi.

2. MATERIAL AND METHODS

2.1. Field sampling

Substrates (foliage, fine branches and wood), soil samples and fungal sporophores were collected in October 2001 and in October 2002 in the state forest of Breuil-Chenue, Nièvre, France in two stands:

– a natural forest stand of beech (*Fagus sylvatica* L., 90% of the stems), oak (*Quercus sessiliflora* Smith, 5% of the stems) and birch (*Betula verrucosa* Ehrh., 5% of the stems);

– a Norway spruce (*Picea abies* (L.) Karst.) stand planted in 1976 after clearfelling of a natural forest stand.

The experimental site of Breuil-Chenue forest is situated in the Morvan Mountains, Burgundy, France (latitude 47° 18' 10", longitude 4° 4' 44"). The elevation is 640 m, the annual rainfall 1280 mm, the evapotranspiration 640 mm and the mean annual temperature 9 °C. The parent rock is granite, containing 23.5% quartz, 44% K feldspath, 28.5% plagioclase, 1.6% biotite and 1.6% muscovite. The soil is an alocrisol, with a pH ranging between 4 and 4.5 [3]. The humus is a dysmoder with three layers (L, F and H) [39]. The nitrogen deposition rate is 15 kg N ha^{-1} y^{-1} (Ranger, personal communication). The Norway spruce plantation was not fertilized.

Mature fungal sporophores were collected in October 2001 in the natural forest stand and in October 2002 in the Norway spruce plantation. Traditional mycological identification methods were used for taxonomic determination. The different species were classified into ecological groups according to the literature (accepted knowledge of ecological niches) and their niches observed in the collecting site [9, 11]. The saprotrophic fungi (SF) were divided into seven groups: fungi living on A_I horizon (ASF), fungi living on decaying needles (NSF), fungi living on decaying strobiles (SSF), litter decaying fungi living on F and H layers (FHSF), wood decaying fungi living on small twigs on the ground (TSF), wood decaying fungi living on dead branches, stumps or trunks (DWSF) and fungi living on dead or living wood (DLWSF).

In the natural stand, sporophores of 47 species were collected at random on a plot of 5000 m^2 in four samples for almost all species: 33 ectomycorrhizal fungi (EMF) and 14 SF including 1 ASF,

Table I. Total C, total N, C/N, $\delta^{13}C$ and $\delta^{15}N$ in foliage, fine branches and stem wood of beech, oak and Norway spruce, Breuil forest (n = 5, ± SD).

Species	Organ	C (%)	N (%)	C/N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Beech	Leaves	47.3	2.6	18	–28.5	–4.2
	Fine branches	49.0 ± 1.5	1.1 ± 0.1	43 ± 4	–26.9 ± 0.1	–4.2 ± 0.4
	Wood	50.8 ± 0.6	0.12 ± 0.02	425 ± 71	–28.1 ± 0.9	–3.5 ± 1.6
Oak	Leaves	48.9	2.7	18	–28.6	–4.1
	Fine branches	48.7 ± 0.6	1.1 ± 0.2	45 ± 9	–28.8 ± 0.9	–4.8 ± 0.6
	Wood	46.3 ± 0.6	0.14 ± 0.09	303 ± 100	–26.4 ± 0.1	–3.7 ± 0.5
Norway spruce	Needles	49.0	1.6	31	–27.1	–2.4
	Fine branches	47.8 ± 0.5	0.8 ± 0.1	62 ± 5	–25.3 ± 0.8	–3.4 ± 0.3
	Wood	47.9 ± 0.5	0.07 ± 0.01	722 ± 69	–24.9 ± 0.9	–3.9 ± 0.6

2 TSF, 3 FHSF, 6 DWSF and 2 DLWSF. Sample sizes (n), were as follows: EMF n = 106, SF n = 35, ASF n = 3, TSF n = 5, FHSF n = 5, DWSF n = 16, DLWSF n = 6. Due to the low rainfall during autumn 2001, the saprotrophic fungi were relatively scarce compared to ectomycorrhizal fungi. *Leotia lubrica* (Scop.: Fr.) Pers. (ASF) was found on naked soil devoid of litter, generally near beech trunks.

In the Norway spruce plantation, sporophores of 37 species were collected at random on two plots of 2500 m^2 in four exemplars for almost all species: 20 ectomycorrhizal fungi (EMF) and 17 SF including 1 ASF, 4 TSF, 5 FHSF, 4 DWSF, 2 SSF and 1 NSF. Sample sizes (n), were as follows: EMF n = 65, SF n = 63, ASF n = 1, TSF n = 15, FHSF n = 20, DWSF n = 12, DLWSF n = 3, SSF n = 8, NSF n = 4.

In both stands a total of 71 species were collected. 34 species were collected only in the natural stand, 24 were collected only in the Norway spruce stand and 13 were collected both in the natural stand and the Norway spruce stand. A total of 336 samples were collected for stable isotope analysis. After cleaning, elimination of sporophores contaminated by worms, drying and grinding, 269 samples were kept for stable isotope analysis.

Some sporophores were dissected in order to compare stable isotope composition among stipe, cap and gills. Beech fine roots, mycorrhizas and external ectomycorrhizal mycelium were collected in October 2002 and washed under a dissecting microscope. External ectomycorrhizal mycelium of *Tricholoma sciodes* was cleaned strand by strand with needles. There were four replicates for each organ or tissue, except for external mycelium (one replicate due to the difficulties of collecting). *Cortinarius* and *Lactarius* mycorrhizas were identified at the genus level by morphotyping. *T. sciodes* mycorrhizas, mycelium and sporophores were identified using molecular methods. Stipes and gills of *T. sciodes* were separately collected.

All samples were first air dried and then dried at 60 °C for 48 h. Except for external mycelium, they were ground to a fine powder using a shaker with agate mortar and agate beads.

2.2. Isotopic analysis

Whole mature sporophores were analyzed. Whenever possible several sporophores were included in each sample. Percent C and N and isotopic composition were determined using an online continuous flow CN analyser (Carlo Erba NA1500) coupled with an isotope ratio mass spectrometer (Finnigan delta S). Values were reported in the standard notation ($\delta^{13}C$ ‰ and $\delta^{15}N$ ‰) relative to Pee-Dee Belemnite for C, using PEF (IAEA-CH-7) as a standard, and relative to atmospheric N_2 for N, using $(NH_4)_2SO_4$ (IAEA-N-1) as a standard. $\delta X = (R_{sample}/R_{standard})\text{-}1) \times 1000$, where R is the molar ratio ${}^{heavy}X/{}^{light}X$.

2.3. Statistical analysis

The analysis of variance for the experimental data was conducted using Sigmastat 3.0 (SPSS Inc., Chicago). Student's t-tests were employed to test for significant differences between saprotrophic and ectomycorrhizal fungi, and One-Way-ANOVA for differences among the different species.

3. RESULTS

3.1. The C/N ratio, $\delta^{13}C$ and $\delta^{15}N$ from the living trees to the soil (Tabs. I and II)

3.1.1. C/N

The average C/N ratio was 18 in beech and oak leaves and 31 in Norway spruce needles. The C/N ratio in fine branches was similar in beech and oak (43 and 45 respectively) and higher in Norway spruce (62); in wood the C/N ratios were 303 (oak), 425 (beech) and 722 (Norway spruce).

The C/N ratio in the natural stand was 30 in the L+F layer and 27 in the H layer. It decreased to 20 in the A horizon and then remained relatively stable along the first 40 cm. In the Norway spruce stand, the C/N ratio was higher in the L+F layer than in the natural stand. The ratios in the two stands were identical in the 0−5 to 15−25 cm horizons, but the ratio at 25−40 cm was lower in the Norway spruce stand than in the natural stand. The higher C/N value of Norway spruce litter was a consequence of low total nitrogen content.

3.1.2. $\delta^{13}C$

The $\delta^{13}C$ values were very similar in living beech leaves, living oak leaves and living Norway spruce needles. The

Table II. Total C, total N, C/N, $\delta^{13}C$ and $\delta^{15}N$ in the humus layers (L+F and H horizons) and in the mineral soil at different depths in the natural stand and in the plantation, Breuil forest (n = 5, ± SD).

Stand	Horizon	C (%)	N (%)	C/N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Natural stand	L+F	47.4 ± 4	1.6 ± 0.2	30	–28.4 ± 0.4	–4.3 ± 1.0
	H	37.8 ± 12	1.4 ± 0.1	27	–28.4 ±0.4	–3.5 ± 1.3
	0–5 cm (A_1)	6.2 ± 1.8	0.3 ± 0.1	20	–28.3 ± 0.8	1.4 ± 1.5
	5–10 cm (A_2)	5.4 ± 1.6	0.3 ± 0.06	19	–28.5 ± 0.5	2.9 ± 1.1
	10–15 cm	3.3 ± 1.1	0.2 ± 0.07	18	–27.7± 0.9	3.7 ± 0.6
	15–25 cm	1.9 ± 0.6	0.1 ± 0.03	21	–27.2 ± 0.8	4.7 ± 0.8
	25–40 cm	1.5 ± 0.4	0.1 ± 0.02	19	–27.5 ± 1.3	4.8 ± 0.9
Plantation	L+F	49.4	0.9	56	–28.0	–2.7
	0–5 cm (A_1)	7.2 ± 0.5	0.4 ± 0.02	20	–27.1 ± 0.3	0.6 ± 0.1
	5–10 cm	3.9 ± 0.5	0.2 ± 0.03	19	–27.0 ± 0.4	2.8 ± 0.4
	10–15 cm	2.7 ± 0.4	0.1 ± 0.02	19	–26.8 ± 0.6	2.9 ± 0.6
	15–25 cm	2.0 ± 0.1	0.1 ± 0.01	18	–27.3 ± 0.8	4.4 ± 0.6
	25–40 cm	0.9 ± 0.2	0.06 ± 0.01	15	–26.7 ± 0.9	5.4 ± 0.4

$\delta^{13}C$ values of fine branches were more variable: oak (−28.8‰), beech (−26.9‰), and Norway spruce (−25.3‰). In living trees, wood $\delta^{13}C$ ranged from −24.9‰ (Norway spruce), −26.4‰ (oak) to −28.1‰ (beech).

In soil, $\delta^{13}C$ varied between −26.8‰ and −28.4‰. In the A_1 and A_2 horizons, soil under Norway spruce displayed a lower $\delta^{13}C$ value than soil under the natural stand.

3.1.3. $\delta^{15}N$

The average $\delta^{15}N$ values were identical in living beech and oak leaves (−4.2‰ and −4.1‰ respectively), while lower in living Norway spruce needles (−2.4‰). The $\delta^{15}N$ values differed little between beech and oak in fine branches and wood. In these two species, wood $\delta^{15}N$ was lower than in fine branches or leaves. In Norway spruce, $\delta^{15}N$ increased from leaves to fine branches and from fine branches to wood. Wood $\delta^{15}N$ did not differ among the three species.

In the two stands, compared to the fresh material, there was no ^{15}N enrichment of the litter, which still displayed a negative $\delta^{15}N$ (−4.3‰ in the L+F layer of the natural stand and −2.7‰ for spruce). Strong ^{15}N enrichment was observed in the A_1 horizon of the two stands. In both stands $A_1\delta^{15}N$ became positive (1.4‰ in the natural stand and 0.6‰ in spruce stand). $\delta^{15}N$ continued to increase in the soil according to the depth, without any significant differences between the two stands. At 25−40 cm depth, $\delta^{15}N$ averaged 5‰.

3.2. Total carbon, total nitrogen, $\delta^{13}C$ and $\delta^{15}N$ of sporophores

The average concentration of total C and total N of sporophores was, respectively, 45% and 4%. The total nitrogen concentration ranged from 1.9% to 7% and the carbon concentration from 35 to 54%. There were no statistically valid differences in the total N and C either between saprotrophic and ectomycorrhizal fungi or between sporophores collected in both stands (Fig. 1A).

$\delta^{13}C$ and $\delta^{15}N$ of sporophores differed significantly between saprotrophic and ectomycorrhizal fungi ($P < 0.001$), although the two groups overlapped both for $\delta^{13}C$ and $\delta^{15}N$. The $\delta^{13}C$ of ectomycorrhizal fungi collected in Norway spruce stand was significantly less negative than $\delta^{13}C$ of ectomycorrhizal fungi collected in natural stand. $\delta^{15}N$ of saprotrophic fungi collected in Norway spruce stand was also significantly more negative than $\delta^{13}C$ of saprotrophic fungi collected in the natural stand (Fig. 1B).

3.2.1. Discrimination among saprotrophic fungi through ^{13}C and ^{15}N natural abundance (Fig. 2)

Two sampled fungal species were common to native and Norway spruce stands: *Armillaria gallica* and *Hypholoma fasciculare*. $\delta^{13}C$ and $\delta^{15}N$ of these two fungal species did not differ between the two stands.

Saprotrophic sporophores displayed a variable $\delta^{13}C$ ranging from −25.6‰ (*Leotia lubrica*) to −18.9‰ (*Hygrophoropsis aurantiaca*). Four groups could be statistically distinguished: the ASF group, the NSF group, the SSF, TSF, DWSF and FHSF group and the DLWSF group. *Leotia lubrica* (ASF) slightly modified the $\delta^{13}C$ of its substrate. In average, its own $\delta^{13}C$ was −25.7‰, while soil organic matter $\delta^{13}C$ ranged from −26.0‰ to −29.5‰. *Micromphale perforans* (NSF) (average $\delta^{13}C$ −24.9‰) modified a little more the $\delta^{13}C$ of its substrate (−28.0‰). *A. gallica* (DLWF) shifted its $\delta^{13}C$ towards −20.0‰, possibly indicating a specific carbon fractionation by lignin or cellulose degradation. Between these three groups, the SSF, TSF, DWSF and FHSF groups displayed a moderate enrichment in ^{13}C relatively to the substrate (average $\delta^{13}C$ −23.0‰).

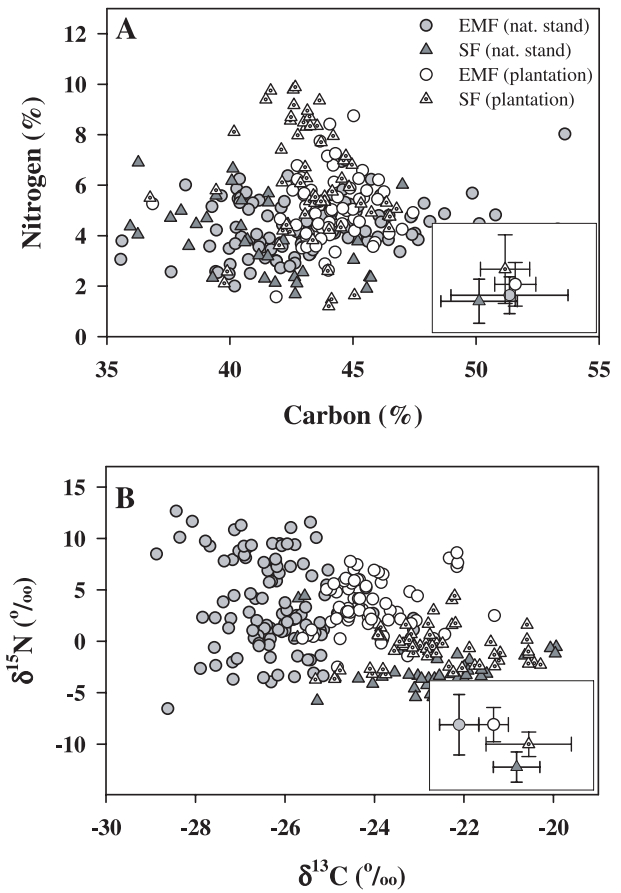

Figure 1. Discrimination among ectomycorrhizal (EMF) and saprotrophic (SF) sporophores collected in the natural stand and in the Norway spruce plantation according to (**A**) total C and total N, and (**B**) $\delta^{13}C$ and $\delta^{15}N$, Breuil forest, (all sporophores, mean and standard deviation for each group). Ectomycorrhizal and saprotrophic sporophores did not differ for total N, nor total C, while they differed for $\delta^{15}N$ ($P < 0.001$) and $\delta^{13}C$ ($P < 0.001$). Ectomycorrhizal fungi in natural stand differed from ectomycorrhizal fungi in Norway spruce plantation for $\delta^{13}C$ ($P < 0.001$), while they did not differ for ^{15}N. Saprotrophic fungi in natural stand differed from saprotrophic fungi in Norway spruce plantation for ^{15}N ($P < 0.001$), while they did not differ for $\delta^{13}C$.

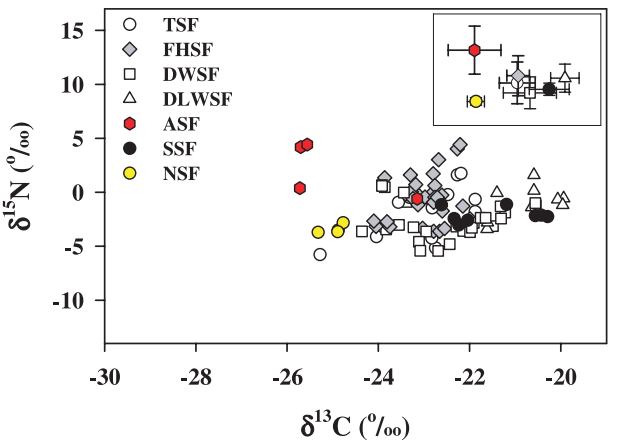

Figure 2. Discrimination among saprotrophic sporophores collected in the natural stand and in the Norway spruce plantation according to $\delta^{13}C$ and $\delta^{15}N$, Breuil forest, (all sporophores, mean and standard deviation for each group). (ASF) saprotrophic fungi living on A_I horizon, (NSF) saprotrophic fungi living on decaying needles, (SSF) saprotrophic fungi living on decaying strobiles, (FHSF) litter decaying fungi living on F and H layers, (TSF) wood decaying fungi living on small twigs on the ground, (DWSF) wood decaying fungi living on dead branches, stumps or trunks and (DLWSF) fungi living on dead or living wood. NSF, TSF, FHSH and DWSF sporophores did not differ for $\delta^{13}C$ nor $\delta^{15}N$. DLWSF sporophores statistically differed from NSF, TSF, FHSH and DWSF sporophores for $\delta^{13}C$ ($P < 0.001$). ASF and NSF sporophores differed from SSF, TSF, FHSH and DWSF sporophores for $\delta^{13}C$ and $\delta^{15}N$ ($P < 0.001$).

Saprotrophic sporophores had a $\delta^{15}N$ ranging from −5.2‰ to 4.5‰. ASF sporophores differed from all the other groups, displaying an average $\delta^{15}N$ of 3.0‰, while the other groups displayed an average $\delta^{15}N$ of −3.0‰.

3.2.2. Discrimination among ectomycorrhizal fungi through ^{13}C and ^{15}N natural abundance

3.2.2.1. Natural stand (Figs. 3A and 3B)

With the exception of one sample of *Cortinarius paleaceus* displaying a $\delta^{13}C$ of −20.7‰, all sporophores of ectomycorrhizal fungi in natural stand had a $\delta^{13}C$ ranging from −23.0‰ (some species of the genus *Lactarius*) to −28.6‰ (one exemplar of *Tricholoma ustale*). $\delta^{15}N$ ranged from −6.5‰ (*Hygrophorus lindtneri*) to 12.7‰ (*Cortinarius alboviolaceus*) with an average of 3‰.

Several species of the genus *Tricholoma* were characterized by a low $\delta^{13}C$ and a large ^{15}N enrichment. Several species of the genera *Cortinarius* and *Hydnum* behaved similarly for ^{15}N, but displayed a low ^{13}C discrimination. The genera *Boletus* and *Xerocomus* behaved similarly with a medium position among the ectomycorrhizal fungi. The genera *Scleroderma*, *Amanita* and *Cantharellus* were relatively close to each other and displayed homogeneous $\delta^{13}C$. The genus *Laccaria* was significantly different from all the other genera with a low ^{15}N discrimination and a large ^{13}C discrimination.

Within genera, individual species displayed distinct signatures (Figs. 4A, 4B and 4C). Species of the genera *Cortinarius* and *Russula* exhibited large variations in $\delta^{15}N$, while species of the genus *Amanita* did not.

There were clearly two different types of ectomycorrhizal fungi displaying small and very large ^{15}N enrichment, respectively. Between these two types, all the intermediaries could be observed.

3.2.2.2. Norway spruce plantation (Figs. 3D and 3C)

In the Norway spruce plantation, sporophores of ectomycorrhizal genera differed little in $\delta^{13}C$ and $\delta^{15}N$, except for the

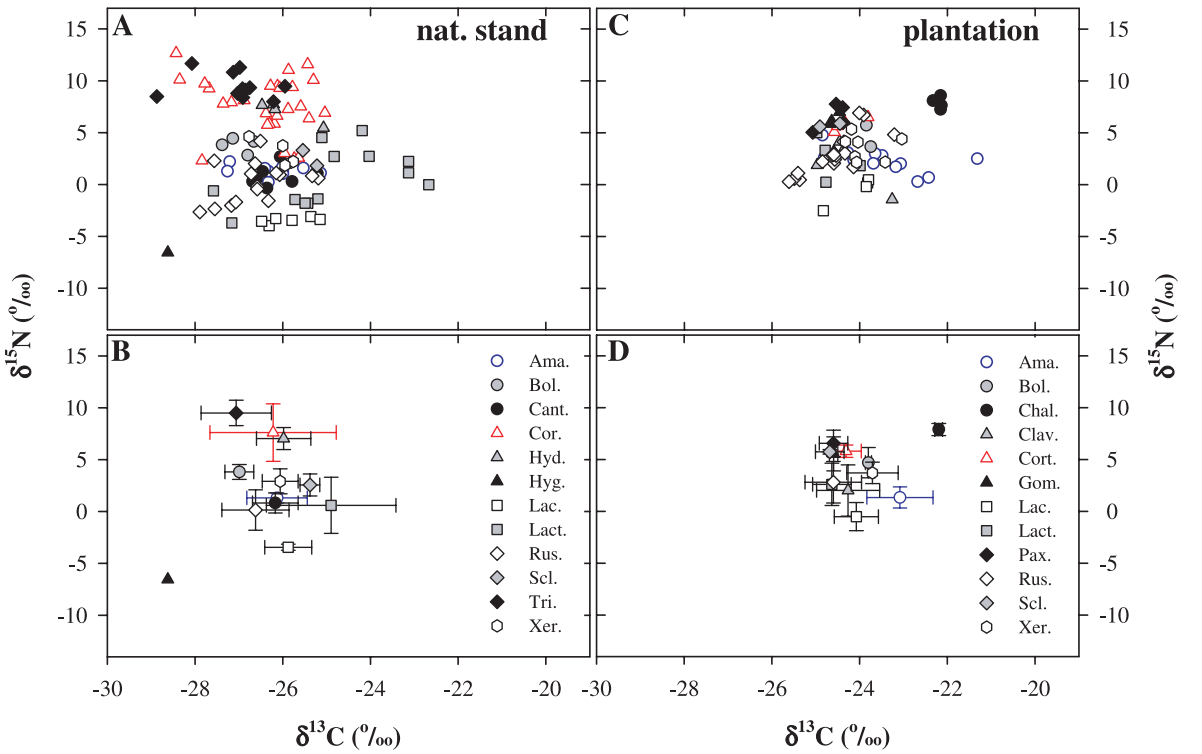

Figure 3. Discrimination among ectomycorrhizal sporophores in the Breuil forest according to $\delta^{13}C$ and $\delta^{15}N$, (**A**) natural stand, all sporophores, (**B**) natural stand, mean and standard deviation for each genus: Ama. = *Amanita*, Bol. = *Boletus*, Cant. = *Cantharellus*, Cor. = *Cortinarius*, Hyd. = *Hydnum*, Hyg. = *Hygrophorus*, Lac. = *Laccaria*, Lact. = *Lactarius*, Rus. = *Russula*, Scl. = *Scleroderma*, Tri. = *Tricholoma*, Xer. = *Xerocomus*, (**C**) Norway spruce plantation, all sporophores, (**D**) mean and standard deviation for each genus: Ama. = *Amanita*, Bol. = *Boletus*, Cant. = *Cantharellus*, Chal. = *Chalciporus*, Clav. = *Clavulina*, Cor. = *Cortinarius*, Gom. = *Gomphidus*, Lac. = *Laccaria*, Lact. = *Lactarius*, Pax. = *Paxillus*, Rus. = *Russula*, Scl. = *Scleroderma*, Xer. = *Xerocomus*.

genus *Chalciporus*, which differed from all others at once by its $\delta^{13}C$ and its $\delta^{15}N$.

Ectomycorrhizal sporophores displayed a $\delta^{13}C$ ranging from −22.2‰ (*Chalciporus piperatus*) to −25.5‰ (*Russula betularum*). $\delta^{15}N$ ranged from −0.6‰ (*Lactarius theiogalus*) to 7.9‰ (*C. piperatus*) with an average of 3.0‰.

3.2.2.3. Comparison between the two stands (Fig. 5)

Overall, $\delta^{13}C$ of ectomycorrhizal sporophores differed significantly between Norway spruce plantation and natural stand, while $\delta^{15}N$ did not (Fig. 5A). All sporophores of ectomycorrhizal species common to both stands displayed a statistically significant $\delta^{13}C$ shift, with the exception of *Scleroderma citrinum* (Fig. 5D). Only *Lactarius theiogallus* and *S. citrinum* shifted significantly in $\delta^{15}N$ between the two stands (Figs. 5B and 5D).

3.2.2.4. Changes in $\delta^{13}C$ and $\delta^{15}N$ from beech fine roots to mycorrhizas and sporophores of ectomycorrhizal fungi (Figs. 6A and 6B)

$\delta^{13}C$ of *Lactarius* mycorrhizas significantly differed from that of beech fine roots whereas *Cortinarius* mycorrhizas did not ($P < 0.001$). $\delta^{15}N$ of *Lactarius* and *Cortinarius* mycorrhizas significantly differed from $\delta^{15}N$ of beech fine roots ($P < 0.001$) (Fig. 6A).

Similarly, $\delta^{15}N$ of *T. sciodes* mycorrhizas significantly differed from $\delta^{15}N$ of beech fine roots (Fig. 6B). The external mycelium of *T. sciodes* mycorrhizas showed increased $\delta^{15}N$ compared to mycorrhizas. But, due to the difficulties of sampling, we had only one replicate. The $\delta^{15}N$ continued to significantly increase from mycorrhizas to sporophore stipes and from stipes to gills. In contrast, the $\delta^{13}C$ showed less discrimination between beech fine roots and *T. sciodes* sporophores than did the $\delta^{15}N$.

4. DISCUSSION AND CONCLUSIONS

In the Breuil forest, as expected, the C/N ratio of Norway spruce foliage was higher than in beech or oak. This ratio also was higher in spruce fine branches and wood than in hardwoods. These differences were reflected in the humus layer. But in the rest of the soil profile no difference was observed between the natural stand and the Norway spruce plantation. Twenty-five years of growth of the spruce were not sufficient to modify soil macro parameters such as total C, total N or C/N ratio. Under both stands, the C/N ratio decreased in the

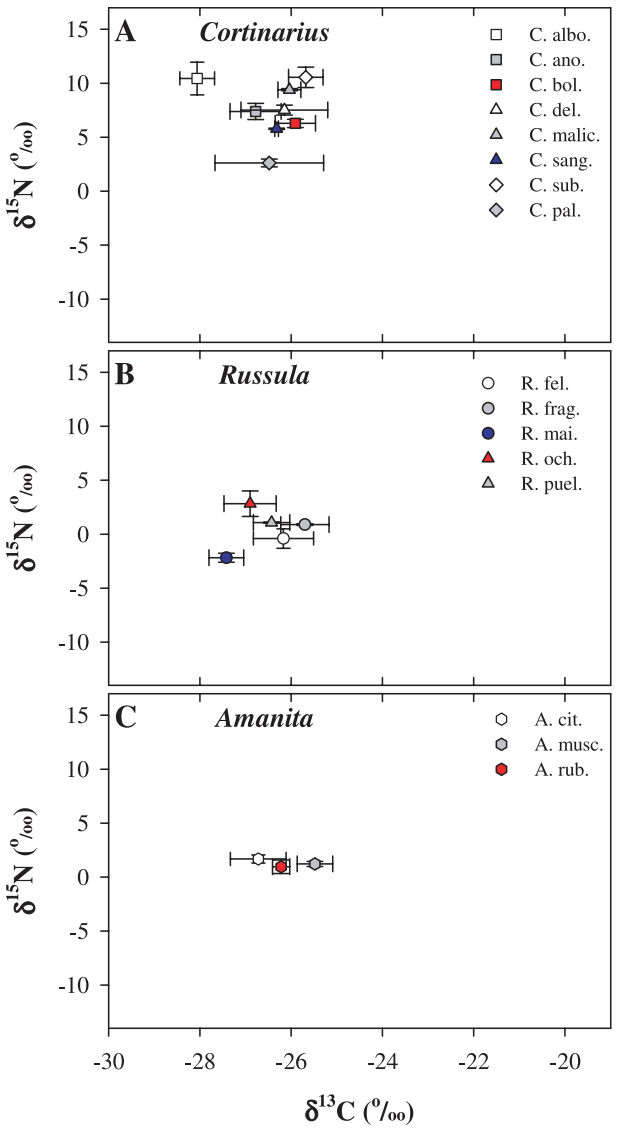

Figure 4. Variations in $\delta^{13}C$ and $\delta^{15}N$ in sporophores of three ectomycorrhizal genera collected in the natural stand: *Cortinarius*, *Russula* and *Amanita*, Breuil forest (mean and standard deviation for each species). C. albo. = *Cortinarius alboviolaceus*, C. ano. = *C. anomalus*, C. bol = *C. bolaris*, C. del. = *C. delibutus*, C. malic. = *C. malicorius*, C. sang. = *C. sanguineus*, C. sub = *C. subtortus*, C. pal. = *C. paleaceus*, R. fel. = *Russula fellea*, R. frag. = *R. fragilis*, R. mair = *R. mairei*, R. och. = *Russula ochroleuca*, R. puel. = *R. puellaris*, A. cit = *Amanita citrina*, A. musc. = *A. muscaria*, A. rub. = *A. rubescens.*

A horizon. Presumably, carbon is lost as CO_2 during decomposition, whereas nitrogen is retained.

$\delta^{15}N$ of total N increased with soil depth without any significant difference between the two stands. $\delta^{15}N$ shifted from −4 to −3‰ in the litter and from 4 to 5‰ in the deeper mineral soil. According to Kendall and McDonnell [40], most soils have $\delta^{15}N$ values ranging from 2 to 5‰. Hobbie et al. [26] reported for Glacier Bay a $\delta^{15}N$ of 0.6‰ in the organic soil and 6‰ in the mineral soil.

The $\delta^{13}C$ of the total C in the soil showed little change with depth; the range was −26.8‰ and −28.4‰. These values are similar to those obtained by Hobbie et al. [26] in the Glacier Bay National Park (Alaska), where the $\delta^{13}C$ ranged from −29.7‰ (*Alnus* foliage) to −27.5‰ in organic soil and −25.6‰ in mineral soil. In the first 15 cm of the Breuil forest soil, $\delta^{13}C$ differed slightly but significantly between the two stands. After 25 years of plantation growth, the Norway spruce seems to have increased $\delta^{13}C$ of soil total C in the upper part of the profile by about 1‰. This may imply that the majority of the soil carbon in the upper profile has been replaced in the 25 years since Norway spruce was planted.

As reported by several authors [18, 23, 26, 38, 41] $\delta^{13}C$ values differ between sporophores of saprotrophic and ectomycorrhizal fungi.

In the Breuil forest, with the exception of *Leotia lubrica*, all sporophores of saprotrophic fungi showed ^{13}C enrichment relative to their substrate. Isotopic ^{13}C fractionation during organic decomposition is not very well known. Cellulose and lignin degradation could be involved in ^{13}C enrichment of sporophores of saprotrophic fungi, although until now no fungal culture studies on known ^{13}C complex substrates have been done.

Most saprotrophic fungi had no or little effect on fractionation of stable N isotopes from their substrates (leaves, twigs or wood). For example, the $\delta^{15}N$ of ASF reflected their substrate (A horizon), which was enriched in ^{15}N in comparison with the litter.

The $\delta^{15}N$ differences observed between saprotrophic sporophores collected in the natural stand and in the Norway spruce plantation could be due to the fact that most of saprotrophic species analyzed in both stands were not the same. These differences could be attributed to differences in isotopic signatures of fungal species.

^{13}C fractionation by sporophores of ectomycorrhizal fungi varied within a narrow range according to the genera and species. For example, *Tricholoma* species did not fractionate C, while species of *Lactarius* were enriched in ^{13}C, less than the purely saprotrophic fungi however. According to the rate of C discrimination in their sporophores, it could be assumed that EM fungi acquire carbon either most exclusively from their host (i.e. *Tricholoma*) or partially from organic matter (i.e. *Lactarius*). This hypothesis is strengthened by the fact that *Lactarius* mycorrhizas displayed ^{13}C fractionation relative to nonmycorrhizal roots, while *Cortinarius* mycorrhizas did not. Handley et al. [19] reported that ectomycorrhizal colonization with *Hydnangium carneum* did not influence $\delta^{13}C$ of *Eucalyptus*. Hobbie and Colpaert [30] shown that colonization of *Pinus sylvestris* by *Suillus* increased overall system $\delta^{13}C$ but not colonization by *Thelephora*.

Overall, the $\delta^{13}C$ of ectomycorrhizal sporophores differed significantly between the Norway spruce and natural stands. These differences were presumably driven by differences in the ^{13}C of recent photosynthates fixed by beech versus Norway spruce and then transferred to ECM fungi.

N fractionation by sporophores of ectomycorrhizal fungi was also very variable. For example, *Hygrophorum lindtneri* did not fractionate nitrogen. In contrast, the genera *Cortinarius*

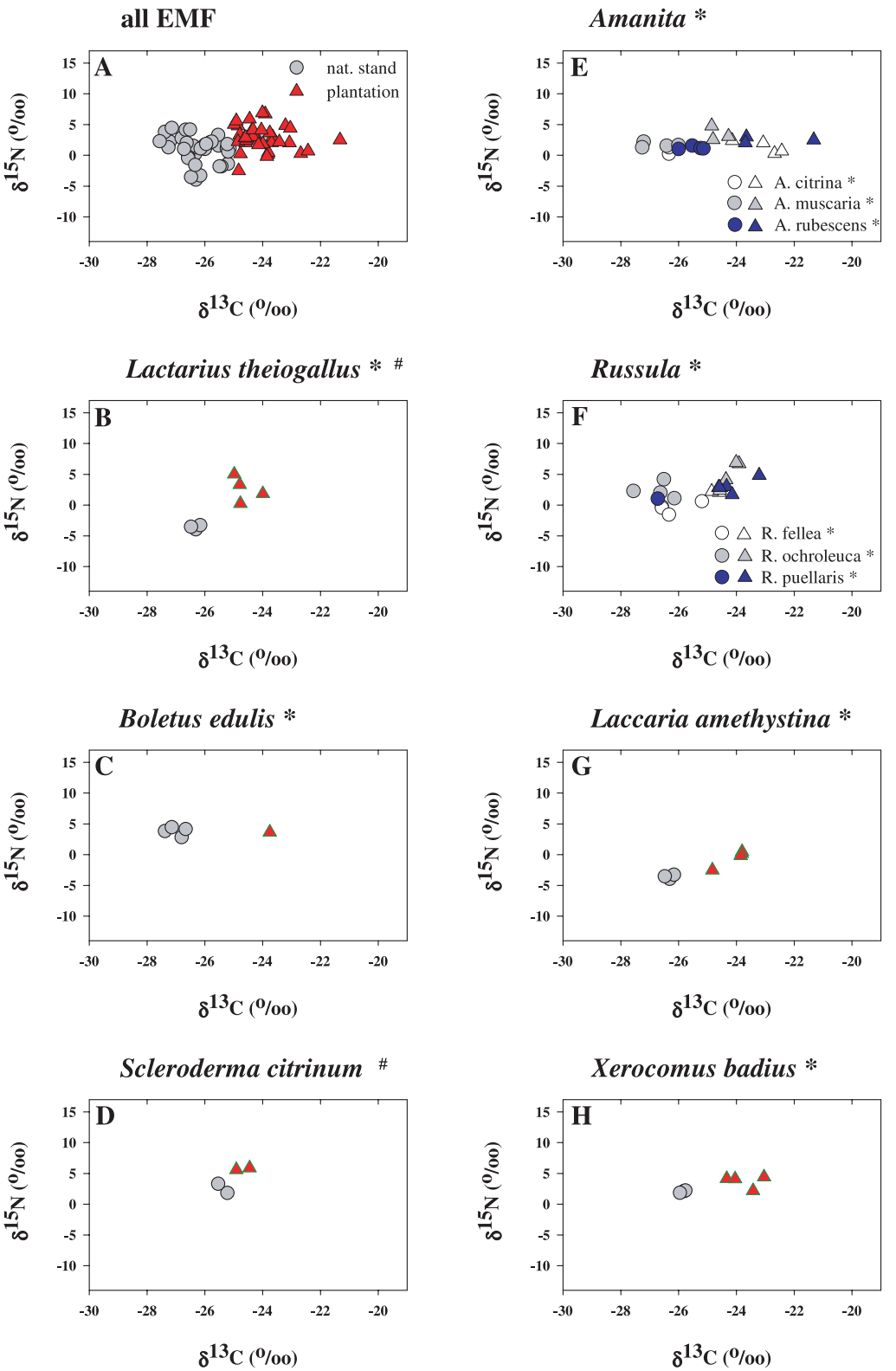

Figure 5. Discrimination among ectomycorrhizal sporophores of 11 species common to natural stand and Norway spruce plantation according to $\delta^{13}C$ and $\delta^{15}N$, Breuil. (**A**) all species; (**B**) *Lactarius theiogallus*; (**C**) *Boletus edulis*, (**D**) *Scleroderma citrinum*; (**E**) *Amanita* (*A. Citrina, A. muscaria, A. rubescens*); (**F**) *Russula* (*R. fellea, R. ochroleuca, R. puellaris*); (**G**) *Laccaria amethystina*; (**H**) *Xerocomus badius*. (all sporophores). * $\delta^{13}C$, differences statistically significant between the natural stand and the plantation ($P < 0.01$) # $\delta^{15}N$, differences statistically significant between the natural stand and the plantation ($P < 0.01$).

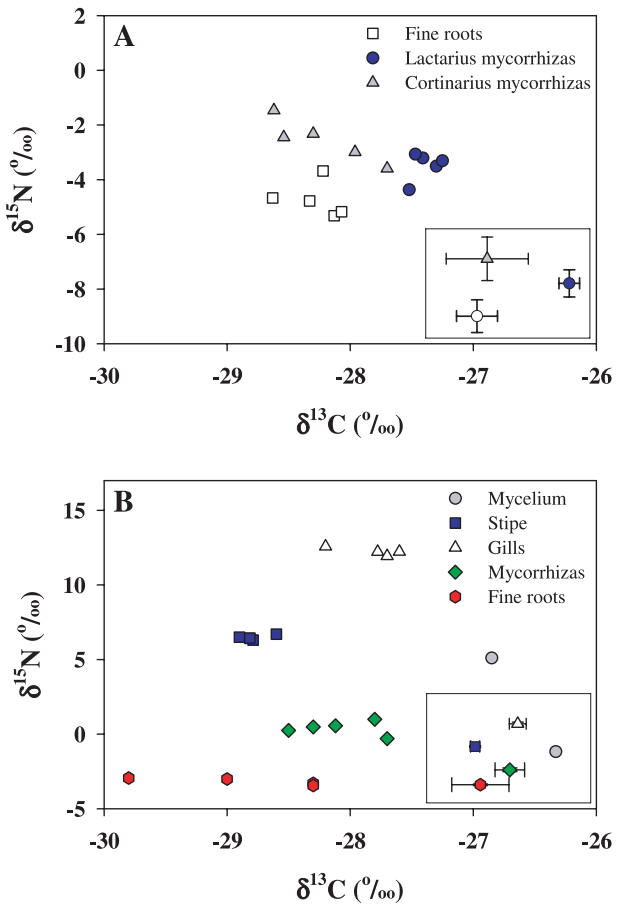

Figure 6. (**A**) Discrimination among beech fine roots, *Lactarius* and *Cortinarius* mycorrhizas according to $\delta^{13}C$ and $\delta^{15}N$ (all samples, mean and standard deviation for each organ). For $\delta^{15}N$, *Lactarius* mycorrhizas and *Cortinarius* mycorrhizas were not different but differed from beech fine roots ($P < 0.03$ and $P < 0.001$). For $\delta^{13}C$, *Lactarius* mycorrhizas statistically differed from beech fine roots and *Cortinarius* mycorrhizas ($P < 0.001$). (**B**) Discrimination among beech fine roots, mycorrhizas, external ectomycorrhizal mycelium, stipe and gills of *Tricholoma sciodes* according to $\delta^{13}C$ and $\delta^{15}N$ (all samples, mean and standard deviation for each organ or tissue). For $\delta^{13}C$, beech fine roots did not differ from mycorrhizas; mycorrhizas differed from stipe ($P < 0.023$) and stipe from gills ($P < 0.05$). For $\delta^{15}N$, all organs or tissues were statistically different from all others ($P < 0.001$). The absence of replicates for external ectomycorrhizal mycelium did not allow statistical calculation for this tissue.

and *Tricholoma* displayed a huge nitrogen fractionation. These results are partly congruent with those of several workers [17, 22–24, 26, 36], who all observed high ^{15}N abundances in ectomycorrhizal fungi sporophores. Ammonification usually causes a small fractionation (+ or – 1‰) between soil organic N and ammonium [37, 40]. This small fractionation due to ammonification cannot explain a shift of 10‰ or 12‰, as observed in *Cortinarius* or *Tricholoma* sporophores. In nitrogen limited systems, like the native forest stand, fractionation by nitrification also is weak. So we can explain ^{15}N enrichment observed in EMF sporophores neither by ammonification nor by nitrification. In ammonium volatilization, the gas has a lower $\delta^{15}N$ than ammonium remaining in the soil [37, 40]. In heavily manured farmland, ammonium volatilization may induce a large increase in $\delta^{15}N$ of the remaining nitrogen. This process cannot be involved in this natural site. Denitrification, which occurs in anaerobic conditions, increases the $\delta^{15}N$ of the residual nitrate, but cannot really be involved in this well drained soil, even if it could occur in the centre of aggregates.

In our study, *Lactarius* mycorrhizas displayed no significant ^{15}N enrichment relative to beech fine roots, while $\delta^{15}N$ of *Cortinarius* and *Tricholoma* mycorrhizas differed significantly from beech fine roots. $\delta^{15}N$ changed from −3‰ (beech fine roots) to −2‰ (*Cortinarius* mycorrhizas) or 0.5‰ (*Tricholoma* mycorrhizas). $\delta^{15}N$ changed from 0.5‰ in *Tricholoma sciodes* mycorrhizas to 4.2‰ in external mycelium. This seems to indicate that for some ectomycorrhizal fungi, enzymatic reactions involved in fungal nitrogen metabolism and transfer to the host could cause a significant $\delta^{15}N$ change, while for other ectomycorrhizal species (*Lactarius*) no change was observed. Mariotti et al. [46] found a small discrimination against ^{15}N during nitrate uptake by 38 species of plants. In general, ammonium or nitrate uptake favours ^{14}N over ^{15}N [37,40]. Bardin et al. [4] found that $\delta^{15}N$ in *Pinus halepensis* mycorrhizas was 2‰ depleted relative to non-mycorrhizal roots. Handley et al. [19] found no difference in N fractionation between mycorrhizal and non-mycorrhizal roots of *Eucalyptus globulus*. In this study, ECM colonization was relatively low, perhaps accounting for lack of difference between none and ECM colonized roots. Högberg et al. [36] found that mycorrhizal roots of Norway spruce and beech were 2‰ enriched in ^{15}N relative to non-mycorrhizal roots. Emmerton et al. [13] showed that *Betula nana* seedlings, which were mycorrhizal with *Paxillus involutus,* displayed no N fractionation when supplied with glutamic acid or glycine but did display significant fractionation against ^{15}N-ammonium. This ammonium fractionation probably occurred during uptake. However, it is very likely that the fractionation occurred because of the quite high concentrations of ammonium available to the mycorrhizas. These concentrations are orders of magnitude higher than those found in natural soils. This line of reasoning, that fractionation only is found when concentrations are very high, has resulted in the general assumption acceptance of the prevailing wisdom (whether true or not): fractionation upon uptake does not occur in N limited systems [29, 32–34]. According to Hobbie et al. [26] and Kohzu et al. [42], the transfer of nitrogen to trees by ectomycorrhizal fungi is a fractionating process, which could occur through amino acid biosynthesis or amino acid transfer to the host.

Our results with *Tricholoma sciodes* also showed that the differentiation processes which led to sporophore formation induced a $\delta^{15}N$ shift. Moreover, inside the sporophores, the process of gill differentiation caused another $\delta^{15}N$ shift. Handley et al. [20] and Taylor et al. [55] obtained similar results. They found a higher $\delta^{15}N$ in fungal caps relative to stipes. Taylor et al. [55] observed a ^{15}N enrichment of protein relative to chitin of about 9‰ in sporocarps relative to hyphae. A preferential export of protein-derived N to sporocarps

and retention of chitin-bound N in mycelium may explain the ^{15}N enrichment in sporocarps [32].

In conclusion, the differences in ^{13}C and ^{15}N natural abundance observed in the Breuil forest among sporophores of saprotrophic or ectomycorrhizal fungi is the result of complex interactions between carbon and nitrogen sources and the different physiological pathways involved in organic matter decomposition, nitrogen uptake, nitrogen assimilation, nitrogen transfer to the host and sporophore differentiation. The host itself has a role on ^{13}C fractionation of sporophores of ectomycorrhizal fungi. Almost all ectomycorrhizal sporophores common to both stands displayed a more negative $\delta^{13}C$ in natural stand than in Norway spruce plantation. This could result from an indirect effect of Norway spruce, through soil organic matter modifications, or from a direct effect of the host, through carbon transfer processes to the fungi. The fact that there was no effect of the host on ^{13}C fractionation of sporophores of saprotrophic fungi is an argument in favour of this second hypothesis.

Our results also show that there is a continuum in ^{13}C and ^{15}N fractionation between ectomycorrhizal and saprotrophic fungi. This could mean that some pathways for carbon and nitrogen acquisition are not different between EMF and SF, while others differ. Among ectomycorrhizal fungi, there seems to be two possible ways of carbon acquisition, partial acquisition from dead organic matter (i.e. *Lactarius*) and acquisition from the host (i.e. *Tricholoma* or *Cortinarius*). Some ectomycorrhizal fungi (*Cortinarius* or *Tricholoma*) seem able to acquire nitrogen from the soil, in inorganic or organic forms, and to supply their host with ^{15}N-depleted nitrogen. Others (i.e. *Lactarius*) seem to have a different way of operating and either supply N to the host without N isotope fractionation or do not supply N to the host. These results are congruent with those of Courty et al. [8], who shown that ectomycorrhizas display in situ differential hydrolytic and oxidative enzymatic activities involved in the decomposition of lignocelluloses, chitin and phosphorus-containing organic compounds. Moreover *Tricholoma* species and probably some other ectomycorrhizal species displayed a significant N fractionation during sporophores differentiation, while saprotrophic fungi did not.

The analysis of ^{13}C and ^{15}N natural abundance in the Breuil forest has allowed differentiation of the main ecological groups of Basidiomycetes present at this site and provided a new insight into the respective trophic role of saprotrophic versus ectomycorrhizal fungi. The processes involved in sporophore differentiation partly explain differences in N fractionation generally observed between ectomycorrhizal and saprotrophic fungi. Moreover, the host has a significant effect on $\delta^{13}C$ of ectomycorrhizal sporophores.

Our objectives were not to investigate the complete nitrogen or carbon cycle in the Breuil forest. However, Hobbie and Hobbie [35] have recently shown that the fractionation against ^{15}N could be used to quantify carbon and nitrogen fluxes in different compartments of natural ecosystems.

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