Ectomycorrhizal fungi introduced with exotic pine plantations induce soil carbon depletion

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Abstract

Exotic pine plantations are promoted for their presumed capacity to provide a net sink of atmospheric C. Millions of hectares worldwide will be subjected to conversion into plantations during the next decades. However, pine introductions are known to result in a marked depletion of soil C, a phenomenon which has remained unexplained. We studied plantations in paramo grasslands of Ecuador, where the effect of the exotic introduction of radiata pines (*Pinus radiata*) and their accompanying ectomycorrhizal fungi can be studied in isolation from other ecosystem disturbances. We suggest that ectomycorrhizal fungi can extract C previously accumulated by paramo grasslands based on (a) a drastic simplification of the ectomycorrhizal community shown by direct DNA identification, (b) a loss of up to 30% soil C within <20 years of plantation, (c) stable C isotope values in fungal fruitbodies which are closer to grassland than pine values, and (d) radiocarbon dating of fruitbodies indicating relatively old C sources for fruitbody formation. Species number in the ectomycorrhizal guild drops to only three fungal species per plantation compared to approximately 100 in comparable native pine stands. Our results provide evidence for a dynamic role of ectomycorrhizal fungi in soil C processing, and question the strategy of introducing pine plantations as a general solution to reduce mounting atmospheric CO_2 levels. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Radiata pine; Plantation; Mycorrhiza; Land-use change; Saprotrophic fungi; Exotic species; Paramo grassland; Carbon mining

1. Introduction

Tree plantations have attracted much attention for their above-ground accumulation of C as tree biomass (Ford-Robertson, 1997; Lee and Dodson, 1996; Maclaren, 1996; Winjum and Schroeder, 1997). With 621 million ha identified globally as "requiring replenishment of forest cover" (Grainger, 1988), tree plantations are expected to represent a major land-use change in the next century. Radiata pine (*Pinus radiata* D.Don) is a species of choice for plantations throughout the world, with 4 million ha already in place and 100,000 ha newly planted each year as an exotic species in the tropics and subtropics (Clapp, 1995; Lavery and Mead, 1998; Sutton, 1999).

However, parallel processes occurring underground have largely been understudied, under the assumption that total soil C would remain unchanged, despite vegetational transformations (Ford-Robertson, 1997; Winjum and Schroeder, 1997). Litter deposition contributes C to superficial soil layers in plantations (Richter et al., 1999), but a net loss of soil C has been consistently documented when the entire soil profile is considered after the exotic introduction of pines and eucalypts, the main plantation species worldwide (Alfredsson et al., 1998; Bashkin and Binkley, 1998; Davis, 1995; Parfitt et al., 1997; Scott et al., 1999; Yeates et al., 1997). While a role for microbes has been invoked by some authors to account for this consistent C loss, no evidence has been produced connecting specific changes in the microbial community with the observed alterations in soil C balances. The most significant microbial change following the establishment of pines or eucalypts in grassland ecosystems is the introduction of ectomycorrhizal (EM) fungi (Allen, 1991). EM fungi are intimately associated with tree roots in plantations, and are necessary for their establishment and survival, while grasslands, dominated by arbuscular-mycorrhizal (AM) fungal species, are typically devoid of such EM fungi (Allen, 1991). The question thus arises as to whether there is a connection between the changes in mycorrhizal status of

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the community resulting from the introduction of EM trees, and the documented loss in soil C contents.

Here we report on a mechanistic connection between a highly simplified, exotic EM guild and the fungally-mediated extraction of soil C. Our study was conducted in the high altitude grasslands of Ecuador. Because these ecosystems receive minimal management, we were able to contrast plantations of various ages directly with immediately adjacent, paired grassland ecosystems, avoiding confounding factors found in highly-managed plantations elsewhere.

2. Materials and methods

2.1. Site description and history

The highlands of the Northern Andes, including most of the Ecuadorean Cordilleras, have been covered in recent geological time by paramo grasslands. Underground, these ecosystems are dominated by arbuscular mycorrhizal (AM) fungi, and no EM fungi are known to occur under natural conditions. Mature andisols under paramo have a deep (up to 3m), C rich B horizon (Jenny et al., 1948; Sturm and Rangel, 1985). While the literature reports up to 30% C contents for these soils, we observed in our sites up to 15% C and 65 kg m^{-2} in the top m of the soil profile (Fig. 1). Organic matter is deposited directly into the soil matrix by the deep grass roots, producing a very homogenous organic horizon which abruptly ends concurrent with the deepest roots.

Beginning in the mid-1970s, radiata pines were introduced into the Ecuadorian paramo as saplings carrying ectomycorrhizal (EM) fungi in their roots. EM infection is achieved at the nursery, where seedlings are grown into soil mixtures containing inoculum from older pines. Saplings and their fungi are planted at 1–3 years of age, 4–5 m apart, in blocks of 1–200 ha. Plantation is performed by hand, without removal of the grassland vegetation, by opening individual holes in the soil only large enough to fit each young sapling (approximately 3000 cm^3). No further management is applied until harvest at 20–30 years. Ecuador plantations are thus different from European and other industrial plantations since disturbance is kept at a minimum; there is no ploughing, furrowing or removal of vegetation prior to planting, and there is no removal of tree material through thinning, pruning or burning. No additions of pesticides or fertilizers are performed in the Ecuador plantations included in this study. Effects of major disturbance are therefore practically negligible, apart from the introduction of the pines and their fungi. Except for plantations in La Esquina, Cotopaxi and Cayambe (see below), which were older than 20 years, plantation canopy had not closed by the time of sampling, and an abundant grass cover was maintained at least until 15 years of plantation. There was no stage in plantation development up to our sampling where soil was not covered by either grass or pine canopies.
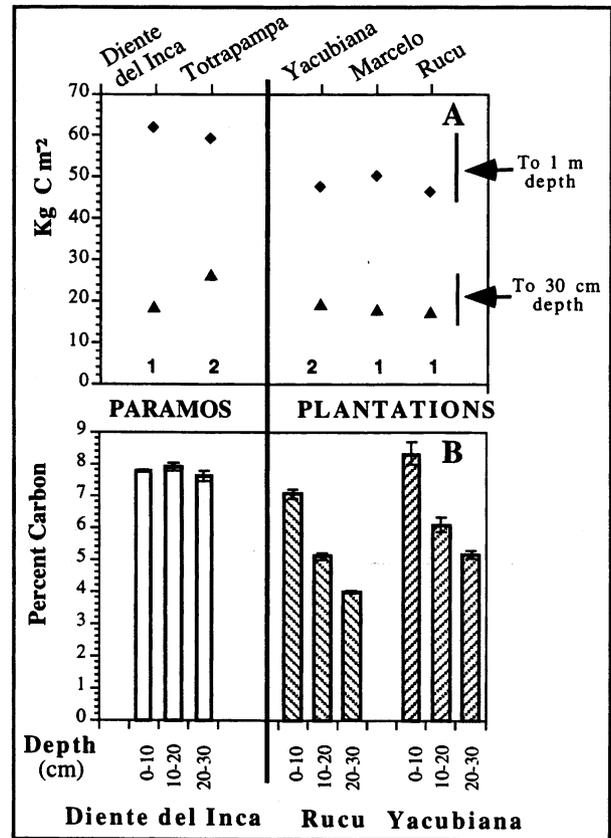

Fig. 1. Carbon contents of soils in paired paramo (left) and pine plantations (right). (A) Total C contents to 30 cm; (▲) and 1 m (♦) depth, corrected for soil bulk density. Paired plantation/paramo plots share a code number. (B) Percent carbon contents at three depths in the top 30 cm of soil profile (mean and SD of three to four independent measurements).

2.2. EM guild description, C contents and isotopic analysis

Samples were obtained from paired paramo/plantation blocks in March 1996 and July/August 1997. For grassland/paramo comparisons (Fig. 1), plots were contiguous and shared the same slope, exposure, soil type and history. Eleven plots were included (after the name of each site the following descriptors are found in parenthesis: latitude S unless otherwise stated; longitude W; altitude in m above sea level; age of plantation in years). *Paramo sites*: Totrapampa (1°24'30"; 79°00'40"; 3700), Diente del Inca (1°21'41.2"; 79°01'13.5"; 3650), Dos Pajaros (1°38'9.2"; 78°51'5.2"; 3650), 3600Arrayan (1°22'46.0"; 3600). *Plantation sites*: Rucu (1°22'23.5"; 79°00'50.0"; 3700; 12), Marcelo 1°21'41.2"; 79°01'13.5"; 3600; 15); Yacubiana (1°24'59.5"; 78°51'13.5"; 3400; 20); 3600ArrayanPl (1°22'46.0"; 79°03'2.5"; 3600; 20); La Esquina (1°31'1.3"; 79°54'20.3"; 3500; 22); Cotopaxi (0°47'; 78°42'; 3400; 25); Cayambe (0°9'24.9" N; 78°03'59.3"; 3300; 25).

For EM analysis, three random, non-edge, 15 cm diam, 30 cm deep soil cores were pooled in every site. Pooling cores is an accepted practice in microbial ecology to overcome technical limitations of culturing methods (Gehring et

al., 1998; Swaty et al., 1998). We pooled cores also to avoid pseudoreplication biases due to the small core sample size and the expected patchy distribution of EM fungi (Bonello et al., 1998). Gehring et al. (1998) and Swaty et al. (1998) experimentally justified the use of pooled samples by showing that across-site variability was overwhelmingly more important than within-site variability in pinion pine forests. It is important to note that the situation in Ecuador plantations is much simplified than in native forests in terms of species number, making the effect of soil core pooling less significant than even Gehring et al. would suggest. In this manner, each plantation represented a single observation/replicate for the estimation of community strucutre and biomass. Pooling of soil cores was performed only for ectomycorrhizal community determinations. Pooling was not performed for C determinations or for stable isotope analyses. More intensive sampling at the Marcelo and Rucu sites included 30 cm cores every 5 m along two orthogonally-oriented 25 m transects in each plantation. Ectomycorrhizae were washed, visually sorted and identified according to published methods (Gardes and Bruns, 1996). Fungus-specific PCR amplification of the rDNA internal transcribed spacer region (ITS) was achieved using primers ITS1-F and ITS4-B, or ITS1-F and ITS4. Amplicons were then identified by restriction fragment length polymorphism (RFLP) analysis using *Alu*I, *Hinf*I and *Dpn*II. ITS-RFLP patterns were matched with those obtained from vouchers of morphologically-identified fruitbodies. No attempt was made at quantifying changes in AM fungi. For unknown basidiomycetes, family placement was conducted using a mitochondrial large subunit rRNA gene database using primer pair ML-5 and ML-6 for amplification and sequencing. Placement of field-derived sequences within a phylogenetic system was achieved by comparison within the database produced by Bruns et al. (1998) using PAUP 3.1.1.

EM biomass was estimated from dry weights of DNA-identified root tips (Fig. 2). To estimate productivity, five 10 × 10 m plots were collected for sporocarps in the Rucu and Marcelo sites. Fresh and oven-dry weights were measured. To compare with the literature, one-time harvests were assumed to represent a 15-day fruiting period (Hedger, 1986). Our conservative estimates for *S. luteus* productivity are of $81–174 \, kg \, ha^{-1} \, yr^{-1}$ (dw), assuming a 10-month fruiting season at April rates, and comparable to Hedger's report of $569–1138 \, kg \, ha^{-1} \, yr^{-1}$ dry weight (dw) on nearby Mt. Cotopaxi (Hedger, 1986). Unpublished data produced for sites near our Rucu and Marcelo plantations by Nathalie D'Andrea over a 2-year period show variability in productivity, with estimates approaching Hedger's highest at their highest and our estimates at the lowest range (D'Andrea, personal communication, 1997). We therefore consider our values to be conservative estimates for actual productivities. Data are given as the mean ± 1 SE unless otherwise noted. To establish statistical significance of differences in proportions data were arcsine transformed and compared using
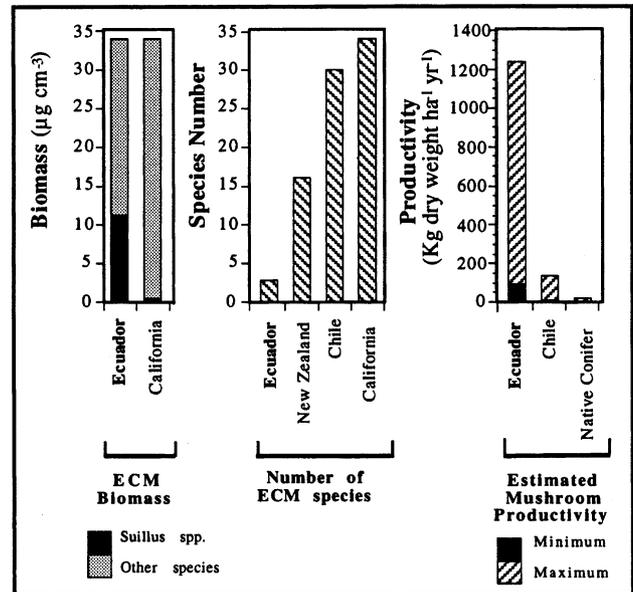

Fig. 2. Ectomycorrhizal (EM) root-tip biomass, number of species and fruitbody productivity in EM guilds in comparable native conifer forests and exotic radiata pine plantations. California values are derived for *P. muricata/Suillus pungens*, and represent conservative estimates (Gardes and Bruns, 1996). California and Ecuador data are based on sporocarp, micromorphology and DNA-identification. Data for radiata pine plantations outside Ecuador are obtained by using sporocarp and micromorphological surveys or only sporocarp data (Chu Chou and Grace, 1988; Dunstan et al., 1998; Garrido, 1986), thus representing an underestimate of actual EM species. Sporocarp productivity for native conifer forests was derived from Vogt et al. (1981, 1992) and Bruns (1995). Statistical confidence limits for Ecuador ECM biomass data (upper s.e./lower s.e. of sin/arcsin-transformed percentage data multiplied by total ECM biomass, in $\mu g/cm^{-3}$, $n = 5$) are as follows: *S. luteus* 18.36/5.10; other species, 29.24/15.30. No comparable statistical analysis is available for other data sets.

unpaired *t*-tests ($P < 0.01$); data were back transformed for graphical presentation. With no permanent plots and open access to local harvesters, it is practically impossible to measure mushroom productivity directly in Ecuador. Estimates of species numbers and biomass comparable to our study are derived from reports of native monospecific forests with *Pinus muricata/S. pungens* in coastal Northern California (Gardes and Bruns, 1996), since the combination *P. radiata/S. luteus* is not known in a native state. Other native conifer forests have more than 50 species in their ECM guild (Vogt et al., 1981, 1992).

Soil pits were dug to 1.9 m for soil analyses in selected sites. Soils were sampled from profiles to a depth of 100 cm for parallel determinations of percent C and bulk density (Lugo and Brown, 1993). Six such paired samples were taken at each pit. Bulk density was determined from the oven-dry weight of soil carefully extracted and kept in a capped, 61.69 cm³ rigid aluminum sleeve to preserve soil structure until weighed. Percent C samples were dried to constant weight in a 60° oven. Depth sampling intervals were 1–10, 10–20, 20–30, 40–60, 60–80, and 80–100 cm, and included a uniform sampling for the entire depth. Roots > 100 μm diam were removed under a microscope. Cleaned soil was then ground

and weighed into tin capsules for analysis. Percent C was measured with an Europa ANCA-NT analyzer. Stable C isotope analysis was completed on triplicate aliquots of soil, plant, and fungal samples on a Europa Scientific 20/20 mass spectrometer with a Europa ANCA-NT C/N analyzer. All carbon isotope measurements are relative to the international V-Pee Dee Belemnite standard by calibration through National Biological Standard #19, and the relative amount of isotopes is expressed as $\delta^{13}C$, in permil (‰) (Galimov, 1985). Analytical precision, determined as the standard deviation obtained on different combustions of the same homogenized sample of standard peach leaves in multiple runs was 0.3‰. Isotopic values for soil samples from 0 to 100 cm depth in the same site were not significantly different from one another. Since it is impossible at this time to access the source of sugar specifically used by mycorrhizae, we assume that the most appropriate value against which fungi should be compared is that obtained for the cambial or mixed needle samples ($\delta^{13}C = -27.9$‰). Radiocarbon content of fruitbodies of *S. luteus*, sampled in July 1997, was determined by Prof. Susan Trumbore at the Lawrence Livermore National Laboratory. This value (+98 ppm) matches the radiocarbon content of atmospheric CO_2 in 1996.

3. Results

Significant losses in C were found in plantations 10 years and older when compared with paired grassland ecosystems without pine plantations (Fig. 1). Careful sampling along the soil profile of Ecuadorian soils showed a density-corrected loss of up to 30% from paramo values already observable 12 years after pine introduction, with a most pronounced C loss in deeper soil layers where EM fungal mycelium was also present (Fig. 1).

Coincident with this C loss, paramo plantations showed an unusually large mushroom productivity for at least one of the introduced EM fungi, *S. luteus* (Fig. 2). *Thelephora terrestris* Fries and *Rhizopogon vulgaris* (Vitt.) M. Lange, also EM fungi, appeared to have equally large productivities in Ecuador plantations, but their fruitbodies, being resupinate or subterraneous, are more difficult to harvest. A third, previously undescribed phenomenon was the extreme reduction in species number of the EM community, with only three species, *S. luteus*, *T. terrestris* and *R. vulgaris* usually found in any single established plantation. This reduction in species numbers was not limited to estimates based on fruitbodies, but was confirmed using DNA identification methods on EM root-tips. Additional species found sporadically elsewhere, and identified on the basis of their mitochondrial DNA sequences included a relative of *Suillus* sp. *Laccaria tortilis* (Bolton) Cooke, was found fruiting in a local nursery.

Isotopic analyses showed that while individual grass and forb species in the paramo varied widely in their $\delta^{13}C$ values, soil organic matter integrated these values at $\delta^{13}C = -24.0 \pm 0.72$‰ (mean $\pm$ SD), with no statistically
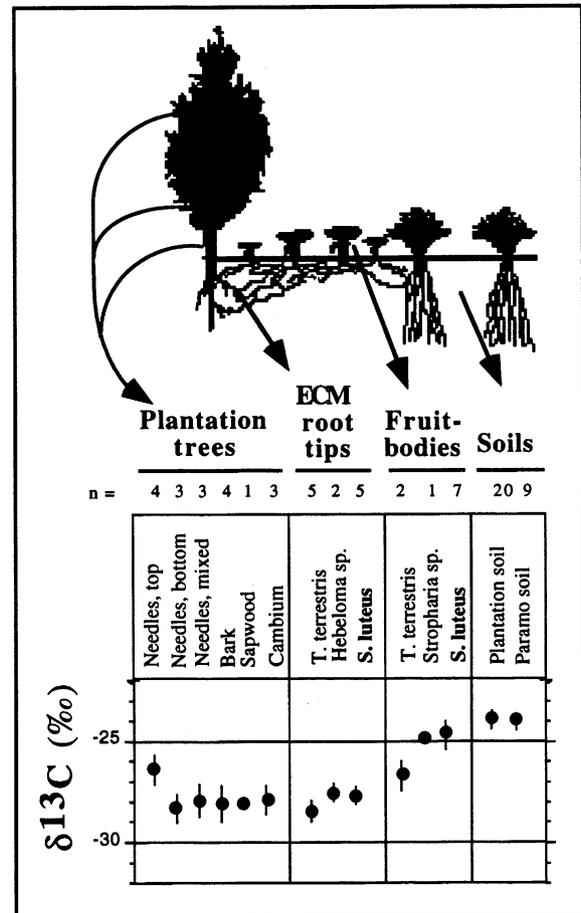

Fig. 3. Stable C isotope analysis of various ecosystem components in radiata pine plantations and adjacent paramo grassland in Ecuador. Mean, SD and sample number are indicated. For *n*, each sample represents multiple measurements for independent sites. Values for soil samples from 0 to 100 cm depth in the same site were not significantly different from one another (Tukey's pairwise comparison after ANOVA, $P > 0.05$). Relatively high variance in cambium and bark are due to varying amounts of woody tissues being inadvertently included. Needle 'mix' was obtained from collections of freshly fallen needles, presumably from various canopy heights and orientations.

significant variation along the soil profile or among sites. Pine tissues, on the other hand, had a comparatively depleted $\delta^{13}C$ value, between -26 and -29‰, typical of a C3 plant (Fig. 3) (O'Leary, 1981). Soils under plantations did not reflect a significant change in stable C isotope composition from the paramo values, except in the litter layer, which was not included as a soil component (Fig. 3). *S. luteus* fruitbodies were found to have a $\delta^{13}C = -24.7 \pm 0.68$‰, closer to soil than to any pine tissue values, from which it was statistically different ($P < 0.001$; Fig. 3). Mycorrhizal root tips identified by DNA analysis as containing *S. luteus* showed a pine-related $\delta^{13}C = -27.6 \pm 0.45$, significantly different from *S. luteus* mushrooms ($P < 0.001$), and undistinguishable from pine tissues alone (Fig. 3). *T. terrestris*, another EM species, showed a $\delta^{13}C$ value closer to that of the host, while a saprotrophic fungus, *Stropharia* sp., growing on a mixture of pine duff and grass straw showed also a mixed $\delta^{13}C$ value (Fig. 3).

Radiocarbon dating of *S. luteus* fruitbodies produced a value (+98 ppm) matching the radiocarbon content of atmospheric CO_2 in 1996. Accounting for dilution with recently fixed C from the pine hosts, we estimated that C used in the formation of *S. luteus* fruitbodies was fixed 2–5 years prior to sampling.

4. Discussion

A loss of soil C observed in the paramo plantations (Fig. 1) is a phenomenon that is generally known when pines are introduced into a variety of native ecosystems elsewhere (Alfredsson et al., 1998; Bashkin and Binkley, 1998; Davis, 1995; Parfitt et al., 1997; Scott et al., 1999; Yeates et al., 1997), although the specific pattern of C loss in deeper soil layers had not been previously described. Although this C loss has not received much attention, several hypotheses have been proposed to account for it. Most of these hypotheses include microbial processing, but no mechanistic connection has yet been established between specific microbial changes and the observed C loss. One of the main difficulties in establishing a mechanistic explanation for this C loss is that plantations are usually accompanied by major ecosystem disturbance in the form of ploughing, furrowing, draining, removal of vegetation cover, fertilization, pesticide application, compaction, pruning, thinning and burning. None of these disturbances are found in the Ecuador plantations we studied, so the simple introduction of the pines themselves and their accompanying EM fungi can be studied in isolation of other confounding factors.

In Ecuador, the loss in soil C associated with plantations coincides with a set of unusual changes that provide a plausible mechanistic connection between the introduction of an exotic fungal flora and soil C loss. First, Ecuador plantations show an exceptionally high mushroom productivity, which is not matched by native forests or plantations elsewhere (Chu Chou and Grace, 1988; Dunstan et al., 1998; Garrido, 1986; Hedger, 1986; Vogt et al., 1981). Productivities of *S. luteus* alone of up to 1×10^6 g ha^{-1} yr^{-1} are up to three orders of magnitude greater than productivities of all EM fungi combined in comparable native forests or plantations elsewhere. This unusual productivity appears incompatible with the poor above-ground performance of Ecuadorian plantations (Acosta-Solis, 1984; Sturm and Rangel, 1985). We therefore hypothesized that the inordinate mushroom productivity observed in these plantations could signal alterations in C processing that made soil C available for EM fungal consumption. Specifically, we suggest that much fungal C can derive from soil sources other than the EM host.

A second observation supporting this hypothesis derives from radiocarbon dating, which showed that fungal fruitbody tissues were conformed at least partly from C fixed from the atmosphere 2 years previous to fruitbody formation, if not before. This observation does not conform with the established view that EM fungi derive their C from recently synthesized simple sugars provided by the host tree. Although it does not appear from the radiocarbon dates that *S. luteus* could access recalcitrant soil C, these data do suggests that at least *S. luteus* was able to derive C from sources relatively older than expected from its EM function. Judging from dates alone, it is plausible that the likely source of C for *S. luteus* might be relatively labile C sources, such as cellulose or other simple organic polymers.

Finally, differences in the natural abundance of stable C isotopes in paramo and plantations allowed us to trace the origins of C found in EM fungal tissues (Fig. 3). In the EM roots, we could not observe any evidence of isotopic fractionation in fungal tissues immediately adjacent to the host root tip, where the ratio of stable isotopes was indistinguishable from the isotopic values of pine needles. Since ectomycorrhizal root tips are known to be formed of at least 40% fungal tissues (Harley, 1971) an isotopic effect caused by the fungi should be reflected in an altered $\delta^{13}C$ value when they are compared with pine tissues. By contrast, $\delta^{13}C$ values of mushroom tissues were closer to those in the paramo soil than the host tissues. Assuming no isotopic fractionation by the fungi, this observation strongly supports the view that these fungi derive at least some of their C from paramo-synthesized sources. These results indicate the involvement of at least one species of EM fungus, *S. luteus*, in the extraction of C from organic matter previously synthesized by the paramo ecosystem.

It must be noted that general surveys of $\delta^{13}C$ values in Northern temperate forests have recently shown that EM fruitbodies tend to be enriched relative to their known hosts (Gleixner et al., 1998; Hobbie et al., 1999; Hogberg et al., 1999; Kohzu et al., 1999). The average $\delta^{13}C$ values found in such studies are coincidentally within range of the values we observed for *S. luteus* in Ecuador. On first consideration, these results would seem to invalidate the assumption made above that EM fungi do not fractionate C isotopes during carbohydrate processing. We have performed laboratory studies with known C sources to address this question and have discovered that (a) isotopic fractionation by EM fungi can occur, but only under specific conditions, (b) fractionation of C isotopes is substrate-dependent, and (c) discrimination between C isotopes on known substrates can vary widely among different fungal species (Henn and Chapela, 2000). In light of our laboratory results, the assumption that all EM fungi will always have a specific $\delta^{13}C$ 'signature' derived from their fractionation of host-tree C compounds cannot be upheld, and instead each ecological situation and fungal species must be considered in detail before any generalization can be made concerning their ecological behavior. In the case of *S. luteus* introduced with radiata pine plantations to the Ecuadorian paramo, the fact that no difference can be observed in $\delta^{13}C$ values between mycorrhizal root-tips and those of pine tissues alone (Fig. 2) strongly suggests that no fractionation can be attributed to the fungus, at least at the root-fungal

interface. The most parsimonious interpretation of our data therefore remains that in the Ecuador situation, the EM fungus *S. luteus* must derive non-host C, explaining both its extraordinary productivity, its stable C isotope composition and its radiocarbon signature.

The capacity of several EM fungi, including *Suillus* spp., to access non-host C has long been suggested (Dighton et al., 1987; Durall et al., 1994; Gadgil and Gadgil, 1971; Zhu et al., 1996). Recent isotope tracing studies have also shown that EM tissues can transport C compounds from one tree species to another, confirming that EM mycelial networks in the soil can act as bidirectional C conduits between the microbial and plant compartments of the ecosystem (Simard et al., 1997a,b). *Suillus* spp. have the enzymatic capacity to break-down and utilize both sugar- and aminoacid-based polymers (Durall et al., 1994). Despite this information, it is still debated whether saprotrophic capacities in EM fungi can be expressed under natural conditions of competition with other, possibly more specialized saprotrophic or EM species (Bending and Read, 1997; Gadgil, 1975). This is indeed a possibility, since we found that roots in exotic plantations in Ecuador typically have only three EM species, as contrasted with native coniferous forests and plantations elsewhere (Chu Chou and Grace, 1988; Dunstan et al., 1998; Vogt et al., 1981), indicating that some release from competitive interactions with other EM species might be in effect in Ecuador (Fig. 2). Under these circumstances, the natural capacity of *S. luteus* to accesses C from saprotrophic growth can be dramatically expressed. There is no doubt that *S. luteus* concurrently supports its growth from host-derived C, as shown by the pine-characteristic distribution of stable C isotopes in mycorrhizal root tips. However, under the Ecuadorian situation the host-fungus association could be best described as a subsidization of fungal growth by the tree. Because fungal biomass production in this case is also dependent on soil-derived sources of C that were accumulated by the paramo grassland in the past, the ecological effect could be described as a 'photosynthesis-subsidized soil C mining'. To what extent this phenomenon is also expressed in more complex situations such as native forests or highly disturbed plantations remains to be assessed.

A mass balance of C processing in Ecuadorean plantations is beyond the scope of this study, but a C loss in the order of 10^4 g m^{-2} observed by us in 20 year-old plantations in the first meter of the soil profile (Fig. 1) is compatible with respiration rates observed for fungi in other temperate grassland ecosystems. Respiration rates of 3.4×10^0 to 2.8×10^{-2} g × (g × d)$^{-1}$, conservatively used as estimates in the field (Moorehead and Reynolds, 1992) could fully account for the observed C losses if a fungal biomass of 4×10^{-3} to 4.89×10^{-1} g × (m^2 × cm)$^{-1}$ was available. Since we observed mycelium down to at least 1.6 m in depth, such a biomass requirement is also compatible with values derived from comparable ecosystems, in the order of 3×10^{-1} g × (m^2 × cm)$^{-1}$ (Moorehead and Reynolds, 1992;

Zak et al., 1994). A mass balance at the ecosystem level might help in elucidating to what extent the loss of C in the soil is offset by the above-ground gains afforded by the accumulation of tree biomass in various ecological scenarios. However such a mass balance or even a model-based approximation would be misleading in our case, since no calibration of models can be effected on the rapidly-changing Ecuadorean situation without a long-term follow-up, no statistically reliable input variables such as total underground biomass, root respiration etc. are available, and existing models have been shown to be sensitive to these limitations (Jenkinson et al., 1999; Smith and Read, 1997).

Finally, we cannot exclude the possibility that a quantitative difference in net primary productivity (NPP) between grassland vegetation and pines could account for a deficit in C inputs to the soil, which would result in a net loss of soil C contents. Nevertheless, this possibility would be contradictory with observations elsewhere, where NPP in pine plantations has been shown to be equal to that of grasslands (Christie and Scholes, 1995; Sparling et al., 1994). Since the studied Ecuador plantations are never denuded of plant cover (grassland or pine), it is also not possible to invoke a transient loss in ecosystem NPP to explain the observed C loss. Our radiocarbon dating of mushroom biomass also militates against this possibility, since a transient drop in C input should be expected to occur in the labile soil C fraction, not the longer-lived fraction accessed by EM fungi in these plantations.

Certain agricultural practices have a net effect of releasing C into the atmosphere from the large pool of soil organic matter (Parfitt et al., 1997; Pennock and Van Kessel, 1997; Wilson, 1978). Our results extend these observations to afforestation scenarios where simplified EM guilds are introduced into AM ecosystems. The observed alterations in fungal community, notably a loss in diversity in an EM guild which is then introduced as an exotic with unexpected effects on C processing in the recipient landscape, provide also a mechanism through which this alteration can occur. Simply stated, ecologically novel biochemical pathways in EM fungi introduced with, and subsidized by, plantation trees, oxidize C pools inaccessible to the AM-driven community. Planting strategies and management of plantations as well as native forests might need to be re-analysed in light of these results.

Acknowledgements

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