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Title: Soil factors influencing ectomycorrhizal sporome distribution in neotropical forests dominated by *Pinus montezumae*, Mexico

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Abstract: Soil factors influencing ectomycorrhizal (ECM) sporome distribution in neotropical forests dominated by *Pinus montezumae* were examined at Sierra Chichinautzin, Mexico. Study sites were located on three volcanoes of different ages and ECM sporomes collected during three consecutive years. *Inocybe* and *Laccaria* species were preferentially found at the youngest site while *Tricholoma* and *Russula* species showed a more abundant distribution at the oldest site. Total sporome richness was negatively correlated with carbon (C) and nitrogen (N) contents in the soil organic horizon. Canonical Correspondence Analysis (CCA) was used to investigate the relationships between sporome species and soil variables. The CCA biplot showed that *Amanita rubescens*, *Inocybe fastigiata* and *Inocybe geophylla* had a strong positive relationship with soil C and N contents, whereas *Inocybe* sp.4 was negatively related to these variables. This indicates an intra-generic variability in fungal responses to soil factors. The measured soil nutrients influenced species composition patterns and the differences in sporome distribution evidenced a large degree of community specialization along the soil quality gradient. Together, these data contribute to a better understanding of the ecology of macrofungi in neotropical forests.

Response to Reviewers: Editor Comment: "I personally think that the supplementary table should be revised to show yearly changes in sporocarp abundance".

Answer: The supplementary table has been modified to show yearly variations in sporome abundance and occurrence (plot frequency).

Reviewers #1 Comments: "I think the methods section would benefit from a specific sub-section that lists all the statistical analyses conducted" and "I think the authors need to clearly specify in the methods which analyses are done at which taxonomic levels and why"

Reviewers #2 Comments: "add logical reason why the present study focused only on epigeous not hypogeous" and "Is not there other ectomycorrhizal tree species other than *Pinus montezumae* at the sites, especially along transects?" and "add mean diameters and heights of adult pine trees" and "State clearly which months you actually investigated" and "add temperature and period for specimen drying" and "I suppose that soil moisture, pH and temperature seem to be basic items for soil measures rather than soil carbon and nitrogen. I wonder why these data were not appeared in the manuscript? Can you refer such the data set from previous papers?".

Answer: The Methods section has been re-written in order to add further details about the study area, the sporome and soil sampling, and the statistical analyses carried out.

a. Study area: additional information is given about the study sites, as suggested by Reviewer #2. The average annual temperature in the area is mentioned, the length of transects between plots has been added and we now cite the names of other ectomycorrhizal hosts that were present at the sites. We also argue that these other tree species were not abundant and that the selected individuals of *Pinus montezumae* were chosen as far as possible from other ectomycorrhizal hosts. We precise that the correlations to unravel the relationships between soil nutrients and ectomycorrhizal species were only implemented with species occurring inside the plots, and not on the transects. Furthermore, average diameter and height of the selected pine trees are now specified.

b. Sporome collection: Sampling months are stated clearly as recommended by Reviewer #2. Reasons for the sampling of epigeous fungi are now given. Hypogeous fungi were not collected because the existing information about hypogeous fungi in the Neotropics is still scarce, which would have constituted a strong limitation for taxonomic determination. Additionally, their sampling requires soil disturbance and because soil samples were to be taken at the same collection sites, we chose to focus on epigeous sporomes only. Temperature and period for sporome drying have been added. The reasons why we focused on rainy seasons only for sporome sampling are explained.

c. Soil sampling: more information about soil horizons is given, with reference for further details to Peña-Ramírez et al. 2009. We also explain that we chose to focus on soil nutrient contents in our study to explain the changes in sporome distribution. This is the reason why we did not measure pH, temperature or moisture at each sampling point. However, variables such as pH or water availability have been measured at each site and we refer to Reverchon et al. (2010b) where they are extensively discussed.

d. Statistical analyses: a specific sub-section describing all the statistical analyses that were conducted has been added to the Methods section. We explain more clearly what were the analyses carried out at the genus-level and those carried out at the species-level, as recommended by Reviewer #1. Only taxonomic distribution (Figure 1) was investigated at the genus-level, to obtain a general pattern of sporome community composition across the chronosequence. Multivariate analyses were carried out at the species-level to examine in detail the relationship between soil nutrients and the distribution of ectomycorrhizal sporomes. We further discuss that fungal genera responses to soil factors are known to vary among species, with references to the studies by Lilleskov et al. 2001; Dickie et al. 2002; Rosling et al. 2003 and Trudell and Edmonds, 2004. We also applied the Bonferroni corrections to the Mann-Whitney and Spearman correlations we carried out. Because some of the correlations were no longer significant after the applied corrections, as predicted by Reviewer #1, we excluded them from the analysis. Instead, we interpreted and discuss the strength of the relationships between soil variables and species data with the equilibrium circle and the goodness of fit values of fungal species to the axes of the CCA biplot (Supplementary figure added).

Reviewer #2 Comment: "Please refer more papers other than Gardes & Bruns (1996) that is rather old"

Answer: more recent references were added such as Richard et al. 2005 and Smith et al. 2007.

Reviewer #1 Comment: "The negative correlation with nitrogen is interesting because other studies have shown the opposite. Was the same trend observed at the root tip level? "

Answer: Further discussion about the negative correlation of species richness with soil total nitrogen content has been written. As suggested by Reviewer #1, we have added more references of studies about nitrogen gradients. We have compared the observed patterns with our observations at the below-ground level and concluded that N might have a stronger effect on aboveground communities (with references to other studies), possibly because of a reduction in resource allocation to sporomes under N-enriched conditions. A more detailed discussion about the differences between the above- and

below-ground ectomycorrhizal communities at our sites, and about the possible reasons for such discrepancies, has been written in Reverchon et al. 2010b.

Reviewer #1 Comment: "The legend for figure 1 should include a list of the genera that were lumped into the "others" category"

Answer: Legend for Figure 1 now includes the list of ectomycorrhizal fungal genera belonging to the "Others" category.

Reviewer #1 Comment: "Figure 3 legend should be clarified that each point is a plot average across three years of data if that is the case"

Answer: Legend for Figure 2 (ex figure 3) has been clarified and correlation lines were shortened.

Reviewer #1 Comment: "Why is CCA axis so long when all the values fall between 2 and -2? I think the figure should be trimmed on this axis so that the space for this figure is used more efficiently"

Answer: The scale of Figure 3 was modified.

Soil factors influencing ectomycorrhizal sporome distribution in neotropical forests dominated by *Pinus montezumae*, Mexico

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Abstract

Soil factors influencing ectomycorrhizal (ECM) sporome distribution in neotropical forests dominated by *Pinus montezumae* were examined at Sierra Chichinautzin, Mexico. Study sites were located on three volcanoes of different ages and ECM sporomes collected during three consecutive years. *Inocybe* and *Laccaria* species were preferentially found at the youngest site while *Tricholoma* and *Russula* species showed a more abundant distribution at the oldest site. Total sporome richness was negatively correlated with carbon (C) and nitrogen (N) contents in the soil organic horizon. Canonical Correspondence Analysis (CCA) was used to investigate the relationships between sporome species and soil variables. The CCA biplot showed that *Amanita rubescens*, *Inocybe fastigiata* and *Inocybe geophylla* had a strong positive relationship with soil C and N contents, whereas *Inocybe* sp.4 was negatively related to these variables. This indicates an intra-generic variability in fungal responses to soil factors. The measured soil nutrients influenced species composition patterns and the differences in sporome distribution evidenced a large degree of community specialization along the soil quality gradient. Together, these data contribute to a better understanding of the ecology of macrofungi in neotropical forests.

Key words

Ectomycorrhizal fungi · Neotropical forests · *Pinus montezumae* · Sporome distribution · Soil quality

1 **Introduction**

2 Ectomycorrhizal (ECM) fungi provide soil resources to their tree hosts and receive
3 carbohydrates in exchange (Göttlicher et al. 2008; Smith and Read 2008) and as such
4 represent an interface between the soil and the host tree (Smits et al. 2008). ECM fungal
5 communities can be assessed through sporome surveys or root tips sampling. Although some
6 authors have considered sporomes as a poor measure of the mycorrhizal status of tree roots
7 (Gardes and Bruns 1996; Richard et al. 2005; Smith et al. 2007), they constitute practical
8 indicators in the estimation of forest fungal biodiversity (Kranabetter et al. 2005; Tóth and
9 Barta 2010) and of soil productivity (Kranabetter et al. 2009a). They can also provide
10 information about fungal characteristics and distribution patterns (Peter et al. 2001a; Baptista
11 et al. 2010). They typically present high species diversity (Richard et al. 2004), their
12 distribution is sensitive to soil quality (Trudell and Edmonds 2004) and to stand age and
13 forest composition (Twieg et al. 2007; Rinaldi et al. 2008). Additionally, they can be
14 accurately identified with proper taxonomic training and can be surveyed across large areas.
15 ECM fruiting bodies were thus used in the present study as a measure of ECM fungal
16 community composition to assess how soil factors influence species distribution.

17 Although many ECM fungi produce conspicuous fruiting bodies (Nara et al. 2003), the
18 species composition of forest macrofungal communities and the factors influencing their
19 distribution are still not well understood (Trudell and Edmonds 2004), especially in tropical
20 regions. Moreover, only a minority of studies has been conducted under natural undisturbed
21 conditions (Erland and Taylor 2002). More information has been generated concerning the
22 influence of soil nitrogen (N) content on ECM fungal communities, due to the increasing
23 presence of anthropogenic N gradients. Both species richness and abundance are affected by
24 an increase in N availability (Lilleskov et al. 2001; Avis et al. 2008) and sporome species

composition changes as well since “nitrophilic” taxa are favoured over “nitrophobic” ones. The relationships between ECM fungal diversity and other soil nutrients have been less investigated, and vary according to the dominant host tree species (Toljander et al. 2006).

In this study, we examined the influence of soil development on the epigeous ECM macrofungi along a soil chronosequence supporting neotropical forests. The dominance of *Pinus montezumae* Lamb. var. *montezumae* in these stands created ideal conditions to minimize the effects of host tree species on the relationships between soil quality and ECM community composition. In a previous study (Reverchon et al. 2010a), we found that species composition of ECM fungal communities as a whole vary according to edaphic conditions. Therefore, we carried out an extensive macrofungi survey in order to analyse in more detail how some selected soil variables were influencing particular fungal species. The contrasts in soil development across the chronosequence generated differences in soil nutrient status and quality: while older sites present a richer and more developed soil profile, young soils are only formed by an organic surface horizon (Peña-Ramírez et al. 2009). Hence, we examined the influence of topsoil quality (total carbon and nitrogen contents, C:N ratio, available phosphorus) on the distribution of specific ECM sporomes. We also investigated the existing relationship between the richness of the ECM sporome community and these soil variables.

Methods

Study area

The study sites have been described in detail elsewhere (Reverchon et al. 2010a, b). Briefly, the Sierra del Chichinautzin Volcanic Field is located at the southern margin of Mexico City.

Three volcanoes with different eruption times and therefore contrasting soil development stages were selected within the protected area Corredor Biológico Chichinautzin: the young Chichinautzin volcano (1835 years B.P.), the middle-aged Guespalapa volcano (4200 years B.P.) and the old Pelado volcano (10,000 years B.P.). Soil properties were analysed extensively (Peña-Ramírez et al. 2009) and soil quality appears to improve with soil development. This volcanic chronosequence therefore forms an ideal framework to investigate the influence of soil parameters on ECM macrofungi composition without the bias of host tree species, age or macroclimate effects.

To establish the sampling plots, one site per volcano was selected in order to minimize the effect of other variables: these sites were located at the same altitude (3100 m) on slopes of less than 10° with a southern orientation, and were covered by natural mature pine forests dominated by *Pinus montezumae*. Since the volcanoes are closely spaced (less than 5 km), climatic conditions in the area are similar: average annual temperatures vary from 10 to 14°C according to elevation, and precipitation ranges from 1000 to 1200 mm per year. Rainfall patterns are strongly seasonal, with 80% occurring during the rainy season, between June and October. Five adult individuals of *P. montezumae* were selected at each site and located at the centre of five sampling plots (10 × 10 m). These sampling plots were separated by transects of approximately 50 m. The selected trees were chosen as far as possible from other ECM trees in order to avoid any interactions with other ECM hosts, although the presence of some individuals belonging to *Abies religiosa*, *Alnus firmifolia* and *Quercus laurina* (Peña-Ramírez, pers. comm.) should be acknowledged. Dendrochronological studies (Peña-Ramírez, unpublished) permitted to select trees of approximately 80 years-old. Tree diameter average was 46 cm at the young site and 62 cm at both the middle-aged and old sites. Tree height averages were 15, 20 and 32 m at the young, intermediate and old site respectively.

Sporome collection and soil sampling

ECM epigeous sporomes were collected weekly within the plots during three whole consecutive rainy seasons (from June to October 2005-2007). No sampling was carried out from November to May because sporome fructification usually occurs during the rainy season in neotropical areas (Garibay-Orijel et al. 2009; Rodríguez-Ramírez and Moreno 2010). Sporomes found on the transects between the plots were also recorded. Hypogeous fungi were not considered in this study because the lack of existing information in Mexico for those species would have constituted a strong limitation for their taxonomical identification, and because of the soil disturbance that their sampling requires. Sporomes were identified based on morphological characteristics, both macroscopic and microscopic (Arora 1986), and fungal genera were determined as ECM following Molina et al. (1992) and Rinaldi et al. (2008). Fungal nomenclature was based on Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>). Voucher specimens were dried at 60°C for two days and placed in the Herbarium of the Laboratorio Microcosmos Bioedáfico, at the Instituto de Geología, UNAM (access Nos. MCCECTC001-MCCECTC2000).

Soil samples were previously collected from a soil profile at each location to determine the general soil characteristics of each study site. The totality of the results are presented in Peña-Ramírez et al. (2009). Soil depth was 35 cm at the young site, with more than 80% of stones after 6 cm, 35.5 cm at the middle-aged site and 200 cm at the old site. The two youngest soils were formed by Ah and AC horizons whereas the old soil, more developed, was formed by Ah, AB, Bw, BC and C horizons.

1 Additionally, during the first rainy season, four soil samples per plot were collected in
2 the soil Ah horizon to establish precise relationships between ECM sporome distribution and
3 topsoil main nutrient contents. Samples were dried and sieved, and total carbon and nitrogen
4 contents were measured through a Perkin Elmer 2400 analyser. Available phosphorus
5 concentrations were determined following the methods described in Bray and Kurtz (1945).
6 These results are summarized in Table 1. Because we chose to focus on how soil main
7 nutrients shaped ECM sporome distribution at the three volcanic sites, other general soil
8 properties such as pH or water availability were not characterized at each sampling point.
9 However, these variables were measured for each soil profile. A gradient in pH and water
10 availability exists across the soil chronosequence, the young site being drier and more acid
11 than the old site. How this gradient may influence ECM community structure is further
12 discussed in Reverchon et al. (2010a).

14 Statistical analyses

16 Differences in main soil nutrient contents between the three sites were assessed with a
17 Kruskal-Wallis test after soil variables resulted non-normally distributed. Post-hoc paired
18 differences were evaluated with a Mann-Whitney U test with a Bonferroni correction for
19 multiple comparisons. Taxonomic distribution was first investigated across the soil
20 chronosequence at the genus-level in order to unravel general patterns of fungal community
21 composition. Moreover, species-level analyses were used to examine in detail the existing
22 relationships between soil variables and the distribution of ECM sporomes. Fungal genera
23 responses to soil factors are seldom straightforward (Lilleskov et al. 2001; Dickie et al. 2002;
24 Rosling et al. 2003) and the effect of nutrient concentrations on fungal distribution, in

particular N content, is known to vary among species, even within the same genus (Trudell and Edmonds 2004). A species-level analysis was therefore the approach chosen to reduce the bias of intra-generic variation. The relationships between soil and fungal variables were carried out using ECM sporomes collected exclusively inside the plots, where soil data were registered. The relationships between total abundance and total richness of the ECM sporome community with the measured soil factors were evaluated with Bonferroni-corrected Spearman correlation tests. Canonical correspondence analysis (CCA) was conducted using the R software (Ihaka and Gentleman 1996) in order to ordinate sites and ECM fungal species based on their sporome abundance. Only dominant taxa occurring at two or more plots were included in the analysis. Equilibrium circles and goodness of fit were used to test the significance of the relationships between community data and environmental parameters.

Results

ECM sporome communities

A total of 2795 sporomes of 153 ECM species were recorded at the three sites during the three consecutive sampling years (Supplementary Table 1). The youngest site presented the largest ECM sporome abundance (1467 sporomes collected during three consecutive years, against 578 for the middle-aged site and 750 for the oldest site) and richness (109 ECM species against 69 for the middle-aged site and 78 for the oldest site).

When analysing ECM sporome community composition at the genus level, the three study sites appeared to be quite different (Fig. 1). *Inocybe* and *Laccaria* sporomes were abundantly recorded across the whole chronosequence, especially at the youngest site, and presented a

1 relatively low proportion of site-exclusive species. On the other hand, *Cortinarius* and
2 *Tricholoma* showed a more definite site-distribution and higher proportions of site-exclusive
3 species (59% and 73% respectively). The largest proportion of site-exclusive species was
4 found at the youngest site (38.5%, against 27.5% for the middle-aged site and 23.1% for the
5 old site).

7 Correlation and multivariate analyses

9 Spearman correlations were used to determine potential relationships between total abundance
10 and richness of the ECM sporome community and the measured soil variables (Table 2).
11 Species richness was significantly and negatively correlated with total C and N contents in the
12 soil organic horizon, indicating that sporome richness was higher at the youngest, less fertile
13 site (Fig. 2).

14 The ordination biplot of CCA analysis (Fig. 3) showed that the communities of ECM
15 sporomes at the 15 sampling plots were not clearly segregated into groups corresponding to
16 the three volcanoes. The first and second axis of the diagram explained 25 and 20% of the
17 variation in fungal distribution respectively. Soil C and N contents were the constraining
18 variables with the highest score for the “x” axis (-0.83, -0.88 and 0.75 for soil C, N and C:N
19 respectively). Species on the left of the biplot and with the highest scores, such as *Amanita*
20 *rubescens* Pers., *Inocybe fastigiata* (Schaeff.) Quél. and *Inocybe geophylla* (Pers.) P. Kumm.
21 have a positive relationship with topsoil C and N contents and a negative one with the C:N
22 ratio of the soil organic horizon, and are therefore more abundant at the oldest site. Soil
23 available P obtained the highest score for the “y” axis of the biplot (0.85), with species to the
24 top, such as *Amanita* sp.1 and *Lactarius salmonicolor* R. Heim & Leclair positively related

1 with P content in the soil organic horizon and therefore associated to the young sites. The
2 equilibrium circle determined that 11 fungal species contributed significantly to the ordination
3 biplot: *A. rubescens*, *Amanita* sp.1, *I. fastigiata*, *I. geophylla*, *Inocybe* sp.1, sp.2, sp.4, sp.7
4 and sp.8, *Laccaria amethystina* Cooke and *L. salmonicolor* (Supplementary Figure 1).

5 Analysing the goodness of fit of these 11 fungal species, we found two different patterns.
6 One group of species contributed significantly to the “C and N” axis, their goodness of fit
7 value being larger for the “x” axis. This group was constituted by *A. rubescens*, *I. fastigiata*, *I.*
8 *geophylla*, *Inocybe* sp.2, sp.4 and sp.7. The second group of species, formed by *Amanita* sp.1,
9 *Inocybe* sp.1 and sp.8, and *L. amethystina*, presented larger values of goodness of fit for the
10 “y” axis and were therefore strongly related to the soil P content. Only *L. salmonicolor* did
11 not belong to either of these groups, its goodness of fit value being larger for a fourth axis of
12 the CCA, constrained by both soil P and C:N ratio.

14 Discussion

16 Total abundance and richness of the ECM sporome community were higher at the youngest,
17 less fertile site. These findings coincide with Baar and ter Braak’s ordination diagram (1996),
18 which suggested that small nutrient and organic matter contents were favourable to ECM
19 fungi. Gehring et al. (1998) also reported that ECM fungal richness did not decrease with
20 lower soil nutrient and moisture levels, which is in agreement with our study since richness
21 was negatively correlated to both soil C and N contents. Many ECM fungi associated with
22 pine species appear to present a negative relationship with soil fertility (Berch et al. 2006;
23 Parrent et al. 2006; Taniguchi et al. 2007) whereas fungi associated with other conifers seem
24 to require richer soils (Toljander et al. 2006; Kranabetter et al. 2009b). *Inocybe* sp.4 was

negatively related to topsoil C and N contents, and species such as *Inocybe* sp.1, *Inocybe* sp.8 and *L. amethystina* were negatively related to soil available P. Species within the same fungal genus responded differently to the measured soil variables, which demonstrates the relevance of species-level analyses to study fungal responses to edaphic factors. *A. rubescens*, for instance, responded positively to total C and N contents in the soil organic horizon whereas *Amanita* sp.1 obtained a very high score on the ordination biplot for its relationship with available P. Molecular data seem to indicate the similarity of *Amanita* sp.1 with *Amanita imazekii* reported by Oda et al. (2001) and further analyses are currently carried out to determine the extent of this similarity. Various studies highlighted the importance of P in shaping ECM community structure (Morris et al. 2008; Twieg et al. 2009). Twieg et al. (2009) also reported a negative relationship between soil available P and ECM fungal richness as a whole, but indicated that the abundance of specific taxa (in that case *Rhizopogon vinicolor* and *Cenococcum geophilum*) was positively related to P. Nutritional requirements of ECM fungi are likely to vary between species, some taxa being better adapted to grow on soils with higher available P content.

Nevertheless, it is difficult to compare our findings with other studies since the gradients in soil fertility they considered to influence species composition are often man-induced (Tarvainen et al. 2003; Rineau and Garbaye 2009). This is especially the case for studies on nitrogen gradients where N levels are much higher than those described here. Despite these discrepancies, we also found that N was negatively correlated with species richness of ECM sporomes, which is in agreement with the findings of other authors (Jonsson et al. 2000; Lilleskov et al. 2001). On the contrary, species richness of root tip communities at the three sites was not affected by N (Reverchon et al. 2010a). This concurs with the results of previous works which report a stronger effect of N on ECM aboveground communities (Jonsson et al.

2000; Peter et al. 2001b; Avis et al. 2003), possibly due to a reduction of resource allocation to sporomes under N-enriched conditions (Lilleskov and Bruns 2001). Species composition of ECM belowground communities is therefore more likely to be related to soil fertility than total abundance or richness (Reverchon et al. 2010a).

Weekly surveys throughout entire sporome production seasons are necessary in order to obtain accurate information about fungal community structure, as suggested by Vogt et al. (1992) and Nara et al. (2003). In this case, sampling during three consecutive whole rainy seasons showed that species composition was different across the soil chronosequence under study. Dominant genera at the youngest and middle-aged sites were *Laccaria* and *Inocybe*, whereas dominant ones at the oldest site were *Amanita* and *Tricholoma*. Multi-site species, such as *Inocybe cookei* Bres. and *Laccaria bicolor* (Maire) P.D. Orton, are useful biodiversity indicators since they could contribute to the resiliency of forest ecosystems, as suggested by Kranabetter et al. (2009a). The differences in the distribution of ECM species determined in this study evidenced a high degree of community specialization along the soil quality gradient. The highest site-exclusiveness for ECM species was recorded at the youngest site, which is similar to what was previously reported for saprophytic fungi (Reverchon et al. 2010b), and was probably enhanced by the spatial heterogeneity and presence of edaphic microniches inherent to young volcanic soils. Site-exclusive species may be suited to particular soil conditions and thus may increase utilization of soil nutrients (Twieg et al. 2009). This may be particularly true at the youngest site where mature pine forests are able to establish despite soil limitations. The examination of ECM root tips from *P. montezumae* at the same study sites (Reverchon et al. 2010a) showed that their larger abundance at the young site is crucial for water uptake, since the relatively small water holding capacity and large evaporation rates at Chichinautzin are limiting factors for tree growth.

Neotropical forests and their associated mycobiota have been scarcely studied (Liang et al. 2007) and the majority of the works carried out in these regions are focused on edible mushrooms only, since mushroom picking is an activity of primordial social and economic importance in Mexico (Zamora-Martínez and Nieto de Pascual-Pola 1995; Garibay-Orijel et al. 2006; Pérez-Moreno et al. 2008; Garibay-Orijel et al. 2009). The determination of fungal species in tropical countries is limited by the fact that many identification keys are made for European or North-American macrofungi, which explains that several taxa could not be identified at the species level. Some of them may even correspond to new, still undescribed, fungal species. This study therefore represents a significant contribution toward a better understanding of the diversity and ecology of macrofungi in neotropical forests.

Species composition of ECM sporome communities is a key factor to analyse the ecological functions played by these fungi, since ECM species reproduction is carried out through fruiting bodies. Sporomes are therefore a relevant indicator for ECM community structure, even when it is important to acknowledge that they constitute only a partial assessment of ECM fungal communities. While previous works have focused on stand age chronosequences to examine the subsequent changes in fungal communities, further research analysing the detailed relationships between soil quality and ECM fungal species would be necessary to generate more information about the dynamics of species distribution in different edaphic conditions. A number of other site properties, such as soil moisture, pH and temperature, or N and P mineralization rates may also influence ECM species composition and would need to be included in the multivariate analysis, for a better understanding of the influence of soil and soil processes on the occurrence of ECM sporomes.

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Figure captions

Fig. 1. Number of collected ECM sporomes per genus and per site (white: 1835 years B.P.; light gray: 4200 years B.P.; dark gray: 10000 years B.P.). “Others” includes the fungal genera *Astraeus*, *Boletus*, *Cantharellus*, *Clavariadelphus*, *Clavulina*, *Entoloma*, *Gomphus*, *Gyrodont*, *Gyromitra*, *Gyroporus*, *Hebeloma*, *Hygrophorus*, *Leccinum*, *Leotia*, *Lyophyllum*, *Porphyrellus*, *Ramaria*, *Sarcodon* and *Suillus*. Black bars represent the number of site-exclusive species

Fig. 2. Total species richness by sampling plot in correlation with a) soil C content (kg m^{-2}) and b) soil N content (kg m^{-2}). R represent Spearman correlation coefficient (*significant at $p < 0.00625$). Each point is a plot average across three sampling years

Fig. 3. CCA ordination biplot of ECM sporome species associated to *Pinus montezumae* based on their abundance at the three study sites during consecutive three sampling years, constrained by soil factors. Letters represent the study sites (C: Chichinautzin; G: Guespalapa; P: Pelado) and numbers represent the sampling plots (five plots per site). Aflavoconia: *Amanita flavoconia* G.F.Atk; Afulva: *Amanita fulva* (Schaeff.) Fr.; Amuscaria: *Amanita muscaria* (L.) Lam.; Arubescens: *Amanita rubescens* Pers.; Asp1: *Amanita* sp.1; Canthracinus: *Cortinarius anthracinus* (Fr.) Fr.; Ccroceus: *Cortinarius croceus* (Schaeff.) Gray; Csp3: *Cortinarius* sp.3; Hmacropus: *Helvella macropus* (Pers.) P. Karst.; Icookei: *Inocybe cookei* Bres.; Ifastigiata: *Inocybe fastigiata* (Schaeff.) Quél.; Igeophylla: *Inocybe aff. geophylla* (Pers.) P. Kumm.; Isp1: *Inocybe* sp.1; Isp2: *Inocybe* sp.2; Isp4: *Inocybe* sp.4; Isp6: *Inocybe* sp.6; Isp7: *Inocybe* sp.7; Isp8: *Inocybe* sp.8; Lamethystina: *Laccaria amethystina* Cooke; Lbicolor: *Laccaria bicolor* (Maire) P.D. Orton; Llaccata: *Laccaria laccata* (Scop.) Cooke; Lsalmonicolor: *Lactarius salmonicolor* R. Heim & Leclair

Table 1. Soil age, classification, and main nutrient contents of the Ah horizon at the three study sites (modified from Reverchon et al. 2010b)

	Chichinautzin	Guespalapa	Pelado
Soil age (years B.P.)	1835 ± 55	2835 ± 75 to 4690 ± 90	9620 ± 160 to 10 900 ± 280
Soil classification (WRB 2006)	Mollic Leptosol	Lepti-vitric Andosol	Eutrisilic Andosol
C (kg·m ⁻²)*	7.68 ± 0.63 ^a	17.8 ± 1.13 ^b	15.4 ± 1.36 ^b
N (kg·m ⁻²) *	0.42 ± 0.03 ^a	1.04 ± 0.05 ^b	0.89 ± 0.06 ^b
C:N*	17.7 ± 0.78 ^a	16.9 ± 0.31 ^a	16.8 ± 0.45 ^a
P (g·m ⁻²) *	2.13 ± 0.37 ^{ab}	2.39 ± 0.29 ^a	1.38 ± 0.19 ^b

*Mean value per site. Four soil samples were collected per plot (5 plots per site) in the Ah soil horizon. Different letters after mean ± S.E. indicate significant differences (Mann-Whitney, p<0.0125)

Table 2. Spearman correlation R values as traducing the relationship between total abundance and richness of ECM sporome species with soil variables

	Soil C content	Soil N content	Soil C:N ratio	Soil P content
	(kg·m ⁻²)	(kg·m ⁻²)		(g·m ⁻²)
Total abundance	-0.491	-0.477	0.272	-0.107
Total richness	-0.734*	-0.670*	0.161	0.045

* Significant correlation at p < 0.00625

Fig. 1.

Figure 2

Fig. 2

Figure 3

Supplementary Table

[Click here to download Supplementary Material: Reverchon et al Supplementary table 1.doc](#)

Supplementary Figure

[Click here to download Supplementary Material: Reverchon et al. Supplementary Figure 1.pdf](#)

Brisbane, May 26th 2011

To: Takashi Yaguchi
Editor in Chief
Chiba University, Japan

Dear Dr. Yaguchi,

I am sending the revised manuscript entitled “Soil factors influencing ectomycorrhizal sporome distribution in neotropical forests dominated by *Pinus montezumae*, Mexico”. This manuscript was first submitted to Mycoscience on February 13th with number MOSC-D-11-00016. It has been carefully reexamined and the recommendations made by the referees have been attended. The modifications made to the manuscript were the following:

1. The supplementary table has been modified to show yearly variations in sporome abundance and occurrence (plot frequency).

2. The Methods section has been re-written in order to add further details about the study area, the sporome and soil sampling, and the statistical analyses carried out.

a. Study area: additional information is given about the study sites, as suggested by Reviewer #2. The average annual temperature in the area is mentioned, the length of transects between plots has been added and we now cite the names of other ectomycorrhizal hosts that were present at the sites. We also argue that these other tree species were not abundant and that the selected individuals of *Pinus montezumae* were chosen as far as possible from other ectomycorrhizal hosts. We precise that the correlations to unravel the relationships between soil nutrients and ectomycorrhizal species were only implemented with species occurring inside the plots, and not on the transects. Furthermore, average diameter and height of the selected pine trees are now specified.

b. Sporome collection: Sampling months are stated clearly as recommended by Reviewer #2. Reasons for the sampling of epigeous fungi are now given. Hypogeous fungi were not collected because the existing information about hypogeous fungi in the Neotropics is still scarce, which would have constituted a strong limitation for taxonomic determination. Additionally, their sampling requires

soil disturbance and because soil samples were to be taken at the same collection sites, we chose to focus on epigeous sporomes only. Temperature and period for sporome drying have been added. The reasons why we focused on rainy seasons only for sporome sampling are explained.

c. Soil sampling: more information about soil horizons is given, with reference for further details to Peña-Ramírez et al. 2009. We also explain that we chose to focus on soil nutrient contents in our study to explain the changes in sporome distribution. This is the reason why we did not measure pH, temperature or moisture at each sampling point. However, variables such as pH or water availability have been measured at each site and we refer to Reverchon et al. (2010b) where they are extensively discussed.

d. Statistical analyses: a specific sub-section describing all the statistical analyses that were conducted has been added to the Methods section. We explain more clearly what were the analyses carried out at the genus-level and those carried out at the species-level, as recommended by Reviewer #1. Only taxonomic distribution (Figure 1) was investigated at the genus-level, to obtain a general pattern of sporome community composition across the chronosequence. Multivariate analyses were carried out at the species-level to examine in detail the relationship between soil nutrients and the distribution of ectomycorrhizal sporomes. We further discuss that fungal genera responses to soil factors are known to vary among species, with references to the studies by Lilleskov et al. 2001; Dickie et al. 2002; Rosling et al. 2003 and Trudell and Edmonds, 2004. We also applied the Bonferroni corrections to the Mann-Whitney and Spearman correlations we carried out. Because some of the correlations were no longer significant after the applied corrections, as predicted by Reviewer #1, we excluded them from the analysis. Instead, we interpreted and discuss the strength of the relationships between soil variables and species data with the equilibrium circle and the goodness of fit values of fungal species to the axes of the CCA biplot (Supplementary figure added).

3. As suggested by Reviewer #2, more recent references were added to “Gardes and Bruns 1996” in the introduction to support that ectomycorrhizal sporome communities are poorly related to ectomycorrhizal root tip communities (Richard et al. 2005; Smith et al. 2007).

4. Further discussion about the negative correlation of species richness with soil total nitrogen content has been written. As suggested by Reviewer #1, we have added more references of studies about nitrogen gradients. We have compared the observed patterns with our observations at the below-ground level and concluded that N might have a stronger effect on aboveground communities (with references to other studies), possibly because of a reduction in resource allocation to sporomes under

N-enriched conditions. A more detailed discussion about the differences between the above- and below-ground ectomycorrhizal communities at our sites, and about the possible reasons for such discrepancies, has been written in Reverchon et al. 2010b.

5. Legend for Figure 1 now includes the list of ectomycorrhizal fungal genera belonging to the “Others” category. Legend for Figure 2 has been clarified and correlation lines were shortened. The scale of Figure 3 was modified.

6. References not validated by cross-checking were reviewed and corrected when necessary.

Following the recommendation to our first submission, we herewith resubmit this paper to Mycoscience. Thank you again for your encouragement.

Sincerely,

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