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Arbuscular mycorrhizal fungi colonize decomposing leaves of *Myrica parvifolia*, *M. pubescens* and *Paepalanthus* sp.

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Abstract Hyphae and vesicles of arbuscular mycorrhizal fungi (AMF) were found within the decomposing leaves of *Myrica parvifolia*, *M. pubescens* and *Paepalanthus* sp. at three montane sites in Colombia. Hyphae, vesicles, and arbuscule-like structures were also found within scale-like leaves of the rhizomes of *Paepalanthus* sp. The litter found in the vicinity of the roots was divided into three decomposition layers. The highest AMF colonization occurred in the most decomposed leaves, which were in close association with roots. In contrast, there were no differences in AMF colonization of roots present in the different decomposition layers. Colonization of decomposing leaves by AMF did not differ between the two closely related species *M. parvifolia* and *M. pubescens*, nor between two sites (Guatavita and Zipacón, Colombia) differing in soil fertility. Occurrence of vesicles in decomposing leaves was correlated with abundant AMF extraradical hyphae among the leaves. We propose that AMF enter decomposing leaves mechanically through vascular tissue. As a consequence, AMF are well positioned to obtain and efficiently recycle mineral nutrients released by decomposer microorganisms before their loss by leaching or immobilization in soil.

Keywords Arbuscular mycorrhizal fungi · Leaf litter · Nutrient cycling · Root proliferation · Tropical montane ecosystems

Introduction

Arbuscular mycorrhizal fungi (AMF) are known to proliferate in organic matter (St. John et al. 1983) and, thereby, to become very well positioned to recycle mineral nutrients efficiently and to reduce losses by leaching (Janos 1983, 1987; Jeffries and Barea 1994). Such tight closure of nutrient cycles by AMF is especially important in highly leached, infertile tropical soils (Herrera et al. 1978; Richards 1996). This prompted Went and Stark (1968a, 1968b) to propose the “Direct Mineral Cycling Theory”, which states that “endotrophic mycorrhiza ... digests cellulose and lignin” and “cycle nutrients ... directly from dead organic matter to the living roots”. However, AMF generally are not regarded as being able to break down complex organic molecules, as they are unable to grow in the absence of a host and produce only small quantities of hydrolytic enzymes (Varma 1999).

Recently, Rivera and Guerrero (1998) found AMF within decomposing leaves of *Myrica pubescens* Humb. & Bonp. ex Willd (Myricaceae), suggesting that these fungi are better positioned to scavenge nutrients than previously thought. Further support for this idea is provided by the association of AMF with decomposing organic particles (St. John et al. 1983) and the presence of AMF in organic “mats” in the canopy (Nadkarni 1981; Maffia et al. 1993; Rabatin et al. 1993). Roots also grow towards aboveground organic matter (Sanford 1987) and such apogeotropic roots can be mycorrhizal (Reddell et al. 1996). In addition, Hodge et al. (2001) demonstrated that the presence of AMF enhanced transfer of nitrogen to host plants from decomposing leaves and accelerated their decomposition.

The main objective of this present study was to extend the observations of Rivera and Guerrero (1998) by determining the extent of AMF colonization within the decomposing leaves of *Myrica parvifolia* Benth, *M. pubescens* and *Paepalanthus* sp. Kunth (Eriocaulaceae) at three different montane sites in Colombia. These species associate with AMF and accumulate mats of dead

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leaves around their stems. Both *Myrica* species associate with the nitrogen-fixing actinomycete *Frankia* sp. (Bravo et al. 1996; Racette et al. 1991). They are small trees or shrubs (2–7 m tall) that grow in eroded or early successional ecosystems of the Andean region of Colombia (Parra 1998). *Paepalanthus* sp. is a rosette plant that grows in páramo grasslands (tropical-alpine ecosystem, 3,000–5,000 m above sea level). The decomposing leaves of this species accumulate aboveground at the base of the rosette, and its apogeotropic roots grow upwards among these leaves. For all three species, mycorrhizal roots are in intimate contact with decomposing leaves. We investigated whether or not AMF colonization of decomposing leaves is affected by degree of decomposition, contrasting soil fertility, or plant species. We also investigated the relationship between AMF colonization of decomposing leaves and AMF structures among litter layers, within roots, and in soil.

Materials and methods

This study was conducted at three montane sites located in the Guatavita, Zipacón, and Subachoque municipalities of Cundinamarca, Colombia. Litter and soil samples were collected from beneath *M. parvifolia* and *M. pubescens* in a deforested and eroded area surrounding the Tominé dam (Guatavita) and at a cattle ranch, Miralejos (Zipacón). We collected *Paepalanthus* sp. samples at the Páramo El Tablazo (Subachoque).

Guatavita is located 2,650 m above sea level at 04°56'23''N, 73°50'11''W, receives 735 mm annual rainfall, and has an annual mean temperature of 15°C (climate information from the Corporación Autónoma Regional, Bogotá). Its eroded soils are clayey, acidic (pH 5.4), and infertile (Bray 2 P 1.7 ppm, organic matter 1.8%; Parra et al. 1985). Zipacón is located 2,550 m above sea level at 04°45'06''N and 74°22'45''W, receives 1,274 mm annual rainfall, and has a mean annual temperature of 14°C. Zipacón's loamy, acidic soils (pH 5.7) are considered relatively fertile (Bray 2 P 7.6 ppm, organic matter 8.4%; Parra et al. 1985). The study site at Zipacón is a cattle pasture that replaced Andean cloud forest but is presently being restored with *M. parvifolia*. El Tablazo is 3,450 m above sea level on the western slope of the Eastern Andes at 04°55'54''N and 74°10'35''W. Its soils are of recent origin and are very acidic (Sturm and Rangel 1985). As is typical of páramos, the turnover rates of litter decomposition and organic matter at El Tablazo are very low.

At Guatavita and Zipacón, litter and the roots it contained were sampled from four 10-cm² quadrats below the canopies of each of 10 *M. parvifolia* and three *M. pubescens* plants. *M. pubescens* was rare at both sites. The quadrats were positioned approximately 60 cm from the trunk on two orthogonal axes with the trunk as their point of intersection. From these quadrats, we also collected soil to a depth of 20 cm for removal of roots.

Litter samples were separated into three distinct layers distinguished by the following characteristics of the leaves: the uppermost layer (top) consisting of entire leaves, the middle layer (middle) with partially fragmented leaves, and the bottom layer (bottom) with highly fragmented leaves closest to the mineral soil and in extensive contact with roots.

In the laboratory, the samples of litter (including roots) from each of the four quadrats were weighed and then thoroughly homogenized and combined. To characterize each litter layer, we used Sigma Scan Pro 5.0 (SPSS Inc.) to measure the areas of 15 leaves or leaf fragments randomly subsampled. Litter dry weight was measured for an additional subsample dried at 80°C for 72 h. One gram of dried leaves from each litter layer was sent to the Instituto Geográfico Agustín Codazzi (Bogotá, Colombia) for

determination of carbon and nitrogen content by dry combustion and gas chromatography using an elemental analyzer (LECO 1000). The remaining litter was stored at 2°C for later assessment of mycorrhizal colonization of leaves and roots.

Subsamples (each 1 g) of leaves and leaf fragments were stained and cleared by a method modified from Phillips and Hayman (1970). Leaves within a litter layer were submerged in KOH for different periods depending upon their tannin contents: top, 25% KOH at 90°C for 12–52 h and 27°C (room temperature) for 32–144 h; middle, 15% KOH at 90°C for 5–36 h and at 27°C for 16–128 h; bottom, 10–15% KOH at 90°C for 3–20 h and at 27°C for 0–64 h. Extremely lignified, intact leaves found only in the top layer were placed in 30% H₂O₂ for 3–15 min. Cleared leaves were acidified with 1% HCl for 1 min and stained in 0.05% acid Trypan blue for 6 h.

To assess AMF colonization in the stained leaves of each subsample, eight randomly selected segments 2 mm wide by 18 mm long were mounted in polyvinyl-lactoglycerol on each of five microscope slides. For each slide, we assessed nine parallel longitudinal transects, orthogonal to the leaf segments and separated by 2.5 mm, using a compound microscope at ×100. Leaves were considered to be colonized by AMF if they contained either coenocytic hyphae with unilateral angular projections (Nicolson 1959) or coenocytic hyphae with terminal vesicles. Septate hyphae of fungi other than AMF were separately recorded. Percent colonization (% total AMF colonization, % vesicle colonization, and % septate hyphae colonization) of leaves was estimated as the number of colonized intersections divided by the total number of observed intersections (approximately 360 per sample).

Root samples (1 g each) were removed randomly from each of the litter and soil samples, cleared and stained (Phillips and Hayman 1970), and mounted on microscope slides for assessment by the magnified intercept method (McGonigle et al. 1990). AMF spores were isolated from a 10-g subsample of litter by wet sieving and decanting (Gerdemann and Nicholson 1963) followed by centrifugal flotation (Jenkins 1964). Extraradical hyphae were extracted from a further 10-g subsample of air-dried litter by the method of Herrera et al. (1986) as follows: after adding 20% H₂O₂, the air dried litter sample was stirred on a magnetic stirrer for 15 min, transferred to a blender and blended five times for 3 s, and then poured through stacked 500-µm and 40-µm sieves. The material was rinsed from the 40-µm sieve on to Sorte 3HW filter paper, air dried for 48 h and weighed. A subsample (0.01 g) was mixed with 2 drops of glycerin on a microscope slide. The number of coenocytic hyphae typical of AMF that intersected four transects (two horizontal and two vertical, separated by 5 mm) on each slide were counted at ×100 magnification using a compound microscope. The length of AMF hyphae in 10 g of dry litter was calculated as: average number of intersections/transect × 1.57 mm/intersection × 20 transects/22 mm square cover slip × weight of material from the 40-µm sieve/0.01 g subsample (modified from Herrera et al. 1986).

At the Páramo El Tablazo, we collected decomposing leaves attached to the rosette bases of four randomly selected *Paepalanthus* sp. plants. These leaves were removed from four equidistant positions around the plants and separated by degree of fragmentation into three layers as described previously for *Myrica*. Leaves were cleared as follows: top and middle, 15% KOH at 90°C for 1 h; bottom, 10% KOH at 90°C for 1–2 h. They were stained, mounted, and the colonization of decomposing leaves and roots (the latter removed only from the bottom layer) assessed as described above for *Myrica*.

For *M. parvifolia*, we used two-way ANOVA and Tukey's HSD post test (Statistix version 7) to evaluate the effects of site and litter layer (with the mineral soil treated as a fourth layer) on AMF colonization parameters and septate hyphae in decomposing leaves, litter dry weight, external hyphae, and AMF colonization in roots. AMF colonization was compared in decomposing leaves and in roots among litter layers by one-way ANOVA and Tukey's HSD post test for *M. pubescens* and *Paepalanthus* sp. Colonization percentages were normalized by arcsine transformation and litter dry weight by log transformation before analysis. As spore counts (made only for *M. parvifolia*) could not be normalized, Kruskal-

Wallis nonparametric one-way ANOVA was used to compare sites and to compare layers within each site separately. Spearman's rank correlation coefficients were computed for all pairwise combinations of variables measured in litter layers for *M. parvifolia*. Significance levels for these correlations were Bonferroni corrected for the number of analyses performed (28) by setting the critical probability level at 0.0018 (ca. 0.05/28) (Scheiner 1993).

Results

Fungi with coarse aseptate hyphae (2.0–12.5 μm in diameter), unilateral angular projections, and terminal vesicles (12.5 \times 15.5 μm to 50 \times 67.5 μm) regularly colonized decaying leaves of *M. parvifolia* (Fig. 1a, b), *M. pubescens* (Fig. 1c) and *Paepalanthus* sp. (Fig. 1d). Total AMF colonization in leaves of the three study species ranged from 1.4 to 97.7%, and percentage of vesicles from 1.9 to 68.6%. Decomposing leaves of *M. parvifolia* and *M. pubescens* also contained AMF spores (Fig. 1e). Thick hyphae (ca. 7 μm diameter) resembling arterial or runner hyphae (see Friese and Allen 1991; Read 1992) consistently colonized midrib veins. Thin hyphae (2.0 μm diameter) originating from the thick hyphae branched successively to colonize lateral veins, the rest of the vascular tissue (Fig. 1f), and mesophyll. Only leaf veins were colonized and vesicles were rare in most of the leaves from the top (least decomposed) layer. AMF also colonized decomposing leaves still attached to the bases of *Paepalanthus* sp. plants, as well as living, scale-like leaves that cover *Paepalanthus* sp. rhizomes (Fig. 1g). Colonization in scale-like leaves was as high as 98.1%, and comprised hyphae, vesicles, and arbuscule-like structures (Fig. 1h).

The percentage total AMF colonization and the percentage of vesicles within the decaying leaves of *M. parvifolia* did not differ between sites, nor was there a

statistically significant interaction between site and decomposition layer. However, colonization did differ between decomposition layers (Table 1). Both variables were significantly higher in the middle and bottom layers than in the top layer. In the middle and bottom layers, leaves were very fragmented (see Table 1, leaf fragment area) and in close contact with roots. Additionally, although similar between sites, C:N ratios decreased from the top to the bottom layer (Table 2). Litter dry weight and extraradical hyphae also differed between decomposition layers (Table 1), both having significantly higher values in the bottom layer than in the top and middle layers.

There was no significant effect of site or decomposition layer on total or vesicle colonization of roots within the litter, nor did colonization variables of roots in the litter differ from those in the underlying soil (Table 1). Total colonization of roots in the underlying soil, however, was higher at Guatavita than at Zipacón (one-way ANOVA, $F_{1,8}$ 6.77, P 0.032). Septate hyphae

Table 2 Percentage of carbon and nitrogen in decomposing *M. parvifolia* leaves sampled from three leaf litter layers located beneath the canopies of *M. parvifolia* plants at two sites. See Table 1 for litter layer designations

Layer	% C	% N	C:N
Guatavita			
Top	41.13	2.01	20.46
Middle	31.93	1.70	18.78
Bottom	23.07	1.42	16.25
Zipacón			
Top	47.71	2.28	20.93
Middle	46.88	2.55	18.38
Bottom	46.30	2.83	16.36

Table 1 Mean values (\pm SD) of variables measured in three leaf litter layers located beneath the canopies of *Myrica parvifolia* plants. Litter layers are designated: top, entire leaves; middle, partially fragmented leaves; bottom, very fragmented leaves, in extensive contact with roots. Differences between leaf litter layers

Layer	Leaf colonization %	Vesicles in leaves %	Septate hyphae in leaves %	Root colonization %	Vesicles in roots %	Spores per 10 g dry litter	External hyphae m per 10 g dry litter	Litter dry weight g/100 cm ²	Area of 15 leaf fragments mm ²
Guatavita									
Top	11.30 \pm 10.42	3.51 \pm 6.64	48.56 \pm 13.67	73.77 \pm 17.39	14.10 \pm 3.71	57.20 \pm 72.28	44.22 \pm 40.31	6.01 \pm 7.21	98.41 \pm 34.42
Middle	31.30 \pm 27.49	14.30 \pm 20.36	53.82 \pm 13.17	58.16 \pm 42.80	21.49 \pm 23.09	103.60 \pm 100.67	91.14 \pm 84.90	7.58 \pm 6.55	54.09 \pm 25.10
Bottom	35.75 \pm 13.32	20.44 \pm 14.71	47.46 \pm 22.89	43.40 \pm 30.75	7.95 \pm 8.85	123.00 \pm 214.75	260.66 \pm 90.6	34.94 \pm 16.64	13.71 \pm 5.87
Soil	NA	NA	NA	50.23 \pm 14.31	11.23 \pm 6.06	ND	ND	NA	NA
Zipacón									
Top	3.73 \pm 3.00	1.30 \pm 2.76	24.52 \pm 6.36	24.71 \pm 5.17	4.34 \pm 3.15	139.60 \pm 112.9	11.35 \pm 6.19	5.23 \pm 1.64	418.83 \pm 248.80
Middle	37.81 \pm 18.18	22.78 \pm 12.66	35.89 \pm 18.39	55.21 \pm 25.69	14.11 \pm 16.37	186.60 \pm 130.6	65.59 \pm 20.66	5.09 \pm 2.79	148.8 \pm 83.88
Bottom	68.54 \pm 23.12	44.54 \pm 21.03	12.87 \pm 16.91	51.84 \pm 24.11	12.54 \pm 13.37	372.00 \pm 194.4	317.64 \pm 40.7	34.50 \pm 14.10	27.73 \pm 16.69
Soil	NA	NA	NA	30.34 \pm 9.03	5.68 \pm 2.39	ND	ND	NA	NA
ANOVA									
Layer	<0.0001*	0.0002*	0.1038	0.5556	0.6730	0.8542	<0.0001*	<0.0001*	ND
Site	0.1925	0.0913	0.0002*	0.0605	0.3460	0.0046*	0.9810	0.7226	ND
L \times S	0.0527	0.2409	0.3779	0.0677	0.4270		0.1464	0.9665	ND

* significant at $P \leq 0.05$

and sites were tested by two-way ANOVAs for all variables except spores and area. Spores were tested for differences between leaf litter layers and between sites by Kruskal-Wallis one-way ANOVAs (NA not applicable, ND not determined)

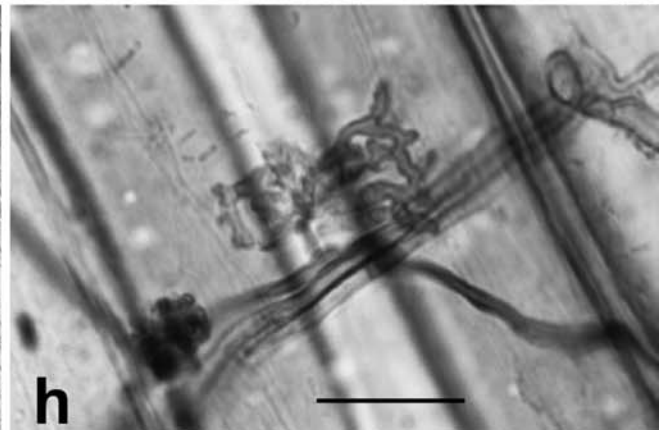
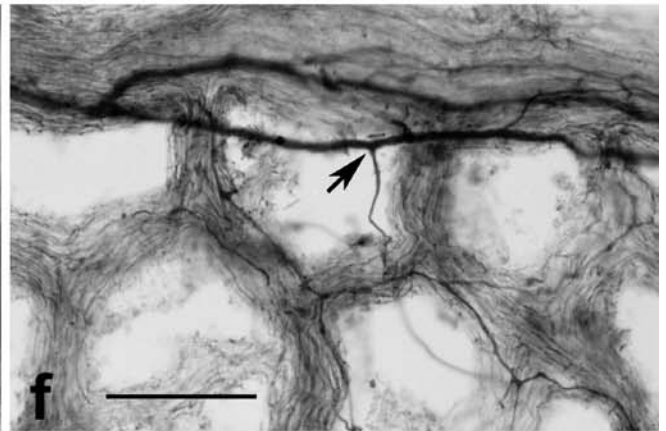
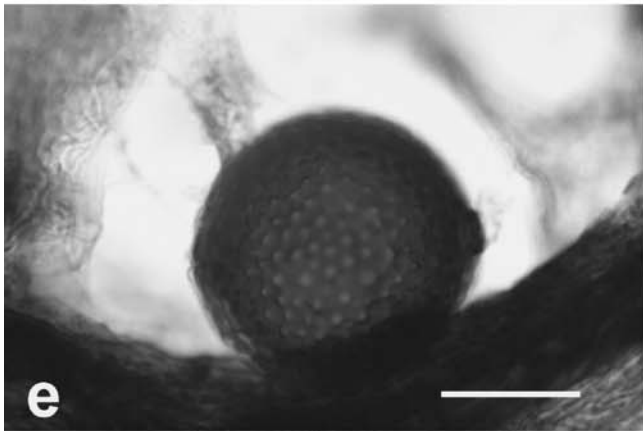
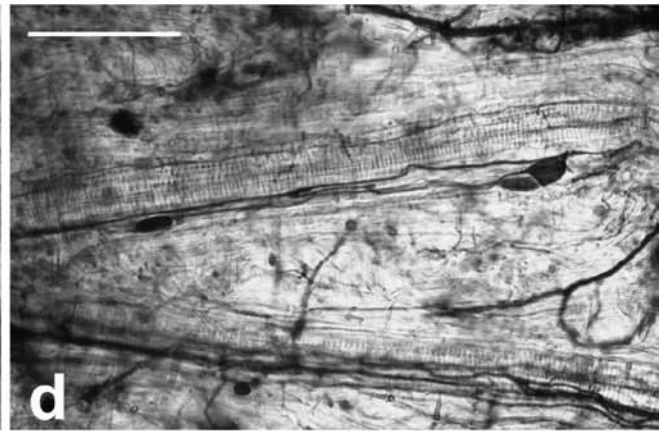
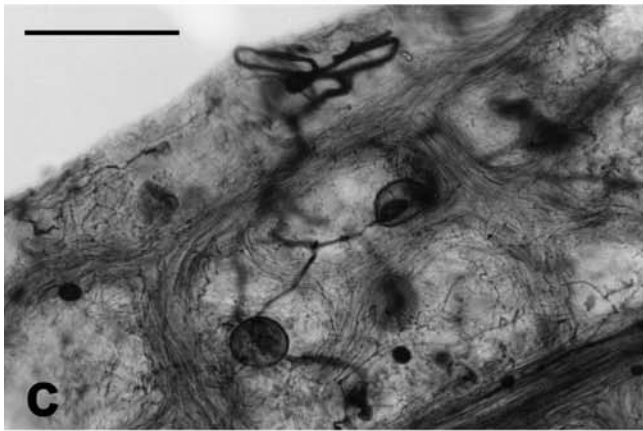
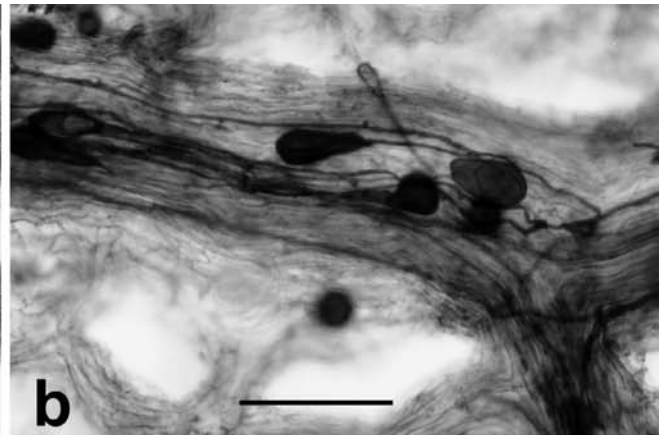
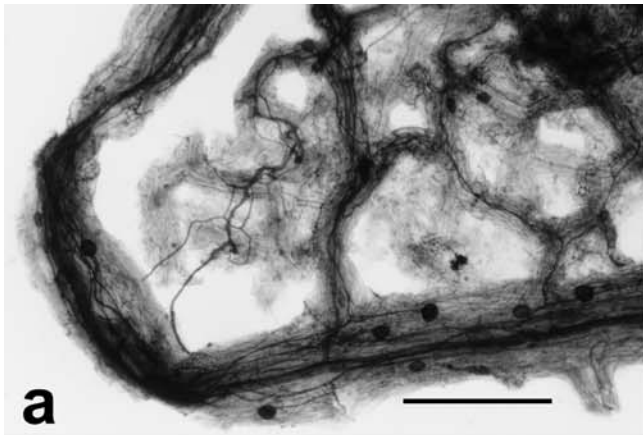


Table 3 Spearman's correlation coefficients and their probabilities (in parentheses) between variables measured in leaf litter layers located beneath the canopies of *M. parvifolia* plants. Significance level was Bonferroni corrected ($P < 0.0018$ for acceptance)

	Leaf colonization	Leaf vesicles	Leaf septate hyphae	Root colonization	Root vesicles	Spores in litter	External hyphae in litter
Leaf vesicles	0.943 (<0.001)*	–	–	–	–	–	–
Leaf septate hyphae	–0.417 (0.061)	–0.498 (0.023)	–	–	–	–	–
Root colonization	0.509 (0.019)	0.418 (0.060)	–0.226 (0.323)	–	–	–	–
Root vesicles	0.520 (0.017)	0.421 (0.058)	–0.232 (0.309)	0.931 (<0.001)*	–	–	–
Spores in litter	0.490 (0.025)	0.606 (0.004)	–0.561 (0.009)	0.012 (0.957)	–0.046 (0.846)	–	–
External hyphae in litter	0.646 (0.002)	0.679 (0.001)*	–0.288 (0.204)	0.177 (0.439)	0.055 (0.810)	0.500 (0.022)	–
Litter dry weight	0.381 (0.089)	0.280 (0.217)	–0.117 (0.613)	0.257 (0.257)	0.246 (0.280)	–0.008 (0.975)	0.664 (0.001)*

* significant at $P < 0.0018$

Table 4 Mean values (\pm SD) of variables measured in three leaf litter layers of *M. pubescens* and *Paepalanthus* sp. plants. See Table 1 for litter layer designations. Differences between decomposition levels were tested by one-way ANOVAs. Mean values in a column followed by different letters are significantly different at $P < 0.05$ (Tukey HSD) (*M* missing i.e. only one of the three samples contained roots, *ND* not determined)

Species	Site	Layer	Leaf colonization %	Leaf vesicles %	Root colonization %	Root vesicles %
<i>M. pubescens</i> (n=3)	Guatavita + Zipacón	Top	1.44 b \pm 1.8	0 b \pm 0	60.48 a (M)	10.18 a (M)
		Middle	37.35 a \pm 24.12	26.89 a \pm 21.21	76.24 a \pm 23.64	37.96 a \pm 20.59
		Bottom	71.90 a \pm 12.15	43.59 a \pm 14.19	78.88 a \pm 10.69	34.08 a \pm 13.78
<i>Paepalanthus</i> (n=4)	El Tablazo	Top	55.78 a \pm 13.18	1.3 b \pm 1.72	ND	ND
		Middle	72.80 a \pm 17.83	5.50 b \pm 8.79	ND	ND
		Bottom	75.68 a \pm 16.16	26.43 a \pm 20.71	66.53 \pm 20.73	ND

colonizing decomposing leaves and the quantity of AMF spores differed significantly between the two sites, but not between decomposition layers. Decomposing leaf colonization by septate hyphae was higher at Guatavita than at Zipacón, but there were more spores in the litter of Zipacón than of Guatavita (Table 1).

Pairwise correlations among variables measured in the leaf litter of *M. parvifolia* across both sites are shown in Table 3. We found significant correlations between total colonization and vesicle colonization for both decomposing leaves and roots in the litter. Extraradical hyphal length was significantly correlated with the percentage of vesicles in decomposing leaves and with litter dry weight.

Fig. 1a-h Arbuscular mycorrhizal fungi (AMF) structures within decomposing leaves of *Myrica parvifolia*, *M. pubescens* and *Paepalanthus* sp., and within scale-like leaves of the rhizomes of *Paepalanthus* sp. **a, b** Hyphae and vesicles within the vascular tissue of decomposing *M. parvifolia* leaves; bars 280 μ m (a), 120 μ m (b). **c** Hyphae and vesicles within a decomposing *M. pubescens* leaf; bar 110 μ m. **d** Hyphae running parallel to xylem and within veins, and vesicles within a decomposing *Paepalanthus* sp. leaf; bar 100 μ m. **e** AMF spore within a decomposing *M. parvifolia* leaf; bar 20 μ m. **f** Thick aseptate hypha with a fine branch hypha arising from a unilateral angular projection (*arrow*) within a decomposing *M. parvifolia* leaf; bar 120 μ m. **g** Hyphae, vesicles, and arbuscule-like structures (*arrows*) within a scale-like leaf of *Paepalanthus* sp.; bar 100 μ m. **h** Arbuscule-like structure within a scale-like leaf of *Paepalanthus* sp.; bar 15 μ m

For *M. pubescens*, decomposing leaf colonization by AMF and colonization of roots in different litter decomposition layers was similar to that observed for *M. parvifolia*. Decomposition layer influenced total leaf colonization and vesicle colonization (Table 4). Both total and vesicle colonization were lowest in the top layer. In contrast, decomposition layer did not influence colonization of roots in the litter. Total decomposing leaf colonization ($F_{1,32}$ 0.15, P 0.698) and vesicle colonization of decomposing leaves ($F_{1,32}$ 1.09, P 0.303) did not differ between *M. parvifolia* and *M. pubescens*. However, *M. pubescens* had higher total ($F_{1,22}$ 9.00, P 0.007) and vesicle colonization ($F_{1,22}$ 16.36, $P < 0.001$) of roots among its decomposing leaves. For *Paepalanthus* sp. there was no significant difference in total leaf colonization between decomposition layers, but vesicle colonization differed between layers (Table 4). It was highest in the bottom (most decomposed) layer. Roots among decomposing leaves of this layer averaged 66.5% total colonization (Table 4).

Discussion

The presence of AMF hyphae and vesicles within decomposing leaves of three different plant species at three different montane sites suggests that this phenomenon is widespread in tropical montane ecosystems where

roots are in intimate association with litter. Roots may grow towards and throughout litter (Sanford 1987), and organic matter can stimulate root proliferation (van Vuuren et al. 1996; Hodge et al. 1999; McKee 2001). Reddell et al. (1996) found mycorrhizal roots proliferating between bark layers of trees in an Australian rainforest. AMF hyphae also are known to proliferate in organic matter (St. John et al. 1983), and AMF spores have been found inside dead seeds, oribatid mites, and even other AMF spores (Rabatin and Rhodes 1982; Taber 1982a, 1982b; Koske 1984). AMF colonization has been reported within decomposing leaves of other montane tropical plant species including *Coffea arabica*, *Inga condonata*, *Musa paradisiaca* (Díaz 2001), *Cedrela montana* (Pabón 2000), and *Myrica cerifera* (C. Aristizábal, unpublished). Hence, AMF hyphae appear to have a well-developed ability to penetrate and thrive in dead organic material.

Our observation that runner hyphae (sensu Friese and Allen 1991; Read 1992) predominantly appear within the midrib veins of top and middle layer decomposing leaves suggests that leaf colonization is initiated through leaf vascular tissue. Leaf veins contain long, wide-diameter tubes (i.e. vessels) within which there are continuous spaces (Hall et al. 1981). We hypothesize that such wide-diameter tubes could provide easy access for fungi such as AMF with limited production of hydrolytic enzymes (Varma 1999). Although AMF typically do not penetrate the vascular tissue of living roots, AMF hyphae and vesicles have been observed in the xylem of *Tradescantia* roots (Taber and Strong 1982) and in the xylem of *Zingiber officinale* scale-like leaves (Taber and Trappe 1982). It is possible that the xylem of the roots examined by those authors was colonized after root death. Taber and Trappe (1982) noted that “hyphae were up to 6 μm wide and in many cases were closely associated with and ran for long distances parallel with the xylem”, supporting vascular tissue as a route for colonization of decomposing plant tissues by AMF.

While we found only sparse hyphae and vesicles in the top layer of decomposing leaves beneath both *M. parvifolia* and *M. pubescens*, colonization was abundant in the middle and bottom layers. Three attributes of the litter could contribute to this pattern. First, leaves in an advanced stage of degradation with many exposed veins may facilitate mechanical penetration by AMF. Second, roots (C. Aristizábal, unpublished) and extraradical hyphae emanating from them proliferate especially in the bottom litter layer where litter dry weight (Table 1) and presumably organic matter mineralization are high. Deep in the litter, high humidity favors growth of saprophytic fungi, thereby stimulating decomposition (Dix and Webster 1995). In contrast, leaves at the surface are scarcely fragmented or compacted and rapidly lose humidity because of their exposure. Third, spread of AMF within the vascular tissue of decomposing leaves may be slow, and leaf fragments in the bottom layer are oldest. The high percentage of vesicles found in leaves in the middle and bottom layers (Table 1) is consistent with

time-dependent colonization because vesicle formation is the final stage of the mycorrhiza colonization cycle (Smith and Read 1997).

The fact that colonization by AMF of *M. parvifolia* decomposing leaves and roots among those leaves did not differ between sites suggests that the conditions affecting colonization of the litter are similar at Guatavita and Zipacón. The similar C:N ratios of the leaf litter at both sites (Table 2) supports this suggestion. In contrast, colonization of roots from the underlying mineral soil differed between sites. Total root colonization was highest at Guatavita, where the soil has lower overall fertility than that of Zipacón. Furthermore, Guatavita has clayey soils that may limit root development and, thereby, increase reliance upon AMF hyphae.

We found significant correlations between extraradical hyphae among litter layers and the percentage of vesicles within decomposing leaves, and between extraradical hyphae and litter dry weight (Table 3). Abbott and Robson (1991) proposed that AMF root colonization depends upon the number of infective hyphae in the soil. We infer that decomposing leaf colonization similarly depends upon the number of extraradical hyphae within the litter. Litter dry weight increases with depth and high organic matter favors abundant extraradical hyphae (St. John et al. 1983).

There was abundant AMF colonization in decomposing leaves of *Paepalanthus* sp. and in subterranean, achlorophyllous, scale-like leaves along *Paepalanthus* sp. rhizomes. Although total colonization of decomposing leaves did not differ between *Paepalanthus* sp. litter layers, the percentage of vesicles was highest among the most decomposed leaves (Table 4, bottom), similar to our findings for *Myrica*. We found arbuscule-like structures (Fig. 1h) in addition to abundant hyphae and vesicles within scale-like leaves below ground. Taber and Trappe (1982) and Imhof (2001) reported AMF hyphae and vesicles (but not arbuscules) in scale-like leaves of *Z. officinale* and *Dictyostegia orobanchoides*, respectively.

The prevalence of vesicles within decomposing leaves (up to 68.6%) suggests that the AMF within those leaves have long persistent connections to host root carbon sources. This inference is supported by high total colonization of decomposing leaves in the bottom layer, where roots are especially abundant, and by the correlation between vesicles in decomposing leaves and the length of extraradical hyphae in the litter. Alternatively, AMF may acquire simple carbon compounds released by the hydrolytic activity of other microorganisms, or AMF themselves may have weak saprophytic ability (Mosse and Hepper 1975; Hodge et al. 2001). The presence of AMF can accelerate the decomposition of organic matter (Hodge et al. 2001), but this could be an indirect effect of AMF enhancement of host nutrition, root exudation, and subsequent effects on decomposer microorganisms (Meyer and Linderman 1986; Linderman 1992).

The occurrence of AMF hyphae and vesicles in decomposing leaves demonstrates that these fungi are very well positioned to scavenge mineral nutrients, even

if mineralization is carried out by other microorganisms. AMF hyphae are likely to interconnect decomposing leaves and live roots among the litter. AMF spores, extraradical hyphae, and colonized roots are abundant in the litter, underlining their importance in nutrient cycling and the need to examine litter when evaluating AMF. In lowland tropical forest, Rose and Paranka (1987) found that roots in litter had higher colonization than those in soil, and Fischer et al. (1994) suggested that AMF spores and roots are missed if the litter is ignored. Furthermore, AMF colonization of roots may differ between soil and litter layers, as we observed. Overall, our findings strongly suggest that AMF are more important for retention and recycling of mineral nutrients in montane tropical ecosystems than previously recognized.

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