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Nitrogen decreases and precipitation increases ectomycorrhizal extramatrical mycelia production in a longleaf pine forest

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Abstract The rates and controls of ectomycorrhizal fungal production were assessed in a 22-year-old longleaf pine (*Pinus palustris* Mill.) plantation using a complete factorial design that included two foliar scorching (control and 95% plus needle scorch) and two nitrogen (N) fertilization (control and 5 g N m^{-2} $year^{-1}$) treatments during an annual assessment. Ectomycorrhizal fungi production comprised of extramatrical mycelia, Hartig nets and mantles on fine root tips, and sporocarps was estimated to be 49 g m^{-2} $year^{-1}$ in the control treatment plots. Extramatrical mycelia accounted for approximately 95% of the total mycorrhizal production estimate. Mycorrhizal production rates did not vary significantly among sample periods throughout the annual assessment (p=0.1366). In addition, reduction in foliar leaf area via experimental scorching treatments did not influence mycorrhizal production (p=0.9374), suggesting that stored carbon (C) may decouple the linkage between current photosynthate production and ectomycorrhizal fungi dynamics in this forest type. Nitrogen fertilization had a negative effect, whereas precipitation had a positive effect on mycorrhizal fungi production (p=0.0292; r^2=0.42). These results support the widely speculated but poorly documented supposition that mycorrhizal fungi are a large and dynamic component of C flow and nutrient cycling dynamics in forest ecosystems.

Keywords Mycorrhizal fungi · Extramatrical mycelia · Extraradical hypha · Biomass · Production

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Introduction

Mycorrhizal fungi mediate carbon (C) and nutrient exchange between plants and soil in forest ecosystems (Treseder and Allen 2000; Hartnett et al. 2004; Godbold et al. 2005; Hobbie 2006). Although previous studies have suggested that mycorrhizal fungi are a large and dynamic component of C flow and nutrient cycles, the majority of these studies have been conducted on fungal species growing either axenically or in association with seedlings in artificial (e.g., growth chambers and glasshouses) environments for relatively short (e.g., weeks to months) periods (Arnebrant 1994; Treseder and Allen 2000; Bidartondo et al. 2001; Staddon et al. 2002, 2003). As a result, the rates and environmental controls of mycorrhizal processes in situ are poorly understood (Wallander et al. 2001; Högberg and Högberg 2002; Treseder 2004; Hobbie 2006). Longer-term, field-based, ecosystem-scale studies of the rates and controls of mycorrhizal production are critical for assessing the role of these fungi in the C flow and nutrient cycling dynamics of forest ecosystems (Norby and Jackson 2000; Treseder and Allen 2000, 2002; Godbold et al. 2005).

Many ecologically and economically important tree species in boreal, temperate, and tropical forests form ectomycorrhizal associations (Fogel 1980; Allen 1991; Hobbie 2006). The fungal component of such associations consists of the following: (1) a Hartig net of fungal mycelia surrounding the cortical cells of many fine root tips, (2) a mantle of fungal mycelia surrounding the surface of these root tips, (3) extramatrical mycelia that radiate from the mantle into the surrounding soil environment, and (4) sporocarp formation (Allen 1991; Wilcox 1991). Comprehensive field-based assessments of ectomycorrhizal production have been hindered by the inability to measure the growth of extramatrical mycelia (Wallander et al. 2001; Högberg and Högberg 2002; Treseder 2004). Consequently, mycorrhizal production assessments in field settings have been typically limited to ectomycorrhizal development on root tips and sporocarp production, despite laboratory-based studies that have documented the quantitative importance of extramatrical mycelia (Colpaert et al. 1992; Arnebrant 1994; Treseder and Allen 2000; Wallander et al. 2001; Staddon et al. 2002).

One promising approach for measuring extramatrical mycelia production of ectomycorrhizal fungi in situ was pioneered by Wallander et al. (2001). In the field, the accrual of fungal-specific biomarkers that break down rapidly after cell death (e.g., ergosterol and signature phospholipid fatty acids; Antibus and Sinsabaugh 1993; Wallander et al. 2001; Gessner and Newell 2002) was assessed using ingrowth mesh bags containing acid-washed sand. Paired sets of ingrowth bags were placed within "closed" (i.e., in polyvinyl chloride [PVC] pipes that prevented the in-growth of roots and ectomycorrhizal fungi) and "open" (i.e., areas that could be colonized by roots and ectomycorrhizal fungi) cores and harvested periodically throughout the year. The biomarker accrual in the closed cores reflected only the growth of saprotrophic fungi, whereas the biomarker accrual in the open cores represented growth of both ectomycorrhizal and saprotrophic fungi. Rates of ectomycorrhizal fungi production were estimated as the difference in biomarker accrual between the open and closed cores (Wallander et al. 2001).

Recently, the utility of the Wallander et al. (2001) ingrowth core approach for measuring rates of extramatrical mycelial production was assessed in a longleaf pine (*Pinus palustris* Mill.) plantation using acid-washed sand and native soil as ingrowth matrixes (Hendricks et al. 2006a). Extramatrical mycelial production of ectomycorrhizal fungi was significantly influenced by the ingrowth matrix, with production rates in the native soil more than 300% higher than corresponding rates in acid-washed sand. Thus, modification of the Wallander et al. (2001) approach to use native soil as the ingrowth matrix may provide estimates of extramatrical mycelial production that better reflect the indigenous rates (Hendricks et al. 2006a). In turn, extramatrical mycelial production assessments via the modified Wallander et al. (2001) approach may be coupled with more traditional assessments of ectomycorrhizal development on root tips and sporocarp formation to provide a more comprehensive assessment of ectomycorrhizal production rates in forest ecosystems.

This study evaluated the rates and ecophysiological controls on in situ ectomycorrhizal fungi production in a 22-year-old longleaf pine plantation. The primary objectives of this study were to (1) assess the various components of ectomycorrhizal fungal production, including the growth of extramatrical mycelia, Hartig nets and mantles on root tips, and sporocarps, (2) compare the temporal production patterns of the various fungal constituents, (3) evaluate how reduced C source strength (i.e., reduced foliar leaf area via scorching and, consequently, decreased current photosynthate production) influences ectomycorrhizal production, and (4) evaluate how increased root C sink strength (i.e., increased soil N availability via fertilization and, consequently, increased fine root and fungal enzyme concentrations and maintenance respiration rates) influences ectomycorrhizal production during an annual assessment.

Materials and methods

Study site

This study was conducted in a 50-ha longleaf pine plantation located at the Joseph W. Jones Ecological Research Center in Baker County, GA (31°15′ latitude, 84°30′ longitude). The plantation was established in 1980 using an approximate 2× 3 m spacing between trees. The closed canopy of the plantation resulted in very little understory vegetation, and a 4% glyphosate herbicide solution was applied to understory plants 1 year before plot establishment (and thereafter as necessary) to ensure that longleaf pine was the only plant species growing in the study plots. Tree heights at the time of study initiation ranged from 10.7 to 13.7 m. The soil in the plantation has been classified as a loamy, siliceous, thermic Arenic Paleudult with weakly developed horizons because of past anthropogenic site use, mixing by fauna, low organic matter content, and lack of silt and clay (Goebel et al. 1997). The climate for this region has been characterized as humid subtropical with an average annual precipitation of 131 cm distributed evenly throughout the year and mean annual low and high temperatures of 9 and 28°C, respectively (Goebel et al. 1997).

Treatments

In the summer of 2001, treatment plots were established in the plantation using two foliar scorching (control and 95%

plus needle scorch) and two N fertilization (control and 5 g N m^{-2} $year^{-1}$) treatments in a complete factorial design yielding four treatment combinations replicated eight times each for a total of 32 plots (see Guo et al. 2004). Each plot was 20×20 m. To minimize potential edge effects, the sample collections were confined to the central 15×15 m subplot of each treatment plot, and the treatment plots were separated by at least a 20-m buffer zone.

In June and July of 2002, after the initiation of first year needle production, foliage scorch treatments were conducted to assess the effects of current photosynthate production, or C source strength, on ectomycorrhizal growth. Approximately 95% of the foliage (i.e., all needles except those on the apical branch of each tree) was removed in 16 randomly selected treatment plots. A 12-m hydraulic lift was used to access the canopy, and a handheld torch connected to a 1,900-l propane tank was used to scorch the foliage for approximately 10 s per needle flush. Previous foliar scorch treatments in the plantation revealed that this method was sufficient to kill foliage without killing branches and terminal buds (Carter et al. 2004). Although this method and degree of defoliation may seem drastic, this treatment has a direct ecological analogue in that longleaf pine occasionally receive and survive 100% crown scorching during prescribed burning events. The hydraulic lift and propane tank were also driven across the non-scorched treatment plots to minimize potential differences in ectomycorrhizal production because of compaction and soil disturbance associated with the scorching process.

The N fertilization treatment was initiated in January 2001 to assess the effects of increasing soil N availability and assimilation, or C sink strength, on ectomycorrhizal production. Consistent with the factorial design, eight foliar scorch and eight nonfoliar scorch treatment plots were randomly selected and fertilized using ammonium nitrate at a rate of 5 g N m^{-2} $year^{-1}$, an approximate twofold increase above N mineralization rates measured in comparable longleaf pine ecosystems (Wilson et al. 1999, 2002). Nitrogen additions were conducted in a manner that tracked natural temporal patterns of N mineralization; the proportion of the 5 g N m^{-2} $year^{-1}$ added each month to the N fertilization treatment plots was based on the percentage of annual N mineralization occurring during that particular month in comparable stands (Wilson et al. 1999, 2002).

Production assessments

Ectomycorrhizal production rates were assessed from February 2002 to February 2003. To obtain comprehensive, ecosystem-scale estimates, extramatrical mycelia, Hartig net, mantle, and sporocarp production were quantified during the annual assessment.

The production of extramatrical mycelia as well as the production of Hartig nets and mantles on fine root tips were measured using a modification of the Wallander et al. (2001) ingrowth core approach (as described in Hendricks et al. 2006a). At the beginning of each sample interval, five sets of "closed" (i.e., 10-cm-diameter × 30-cm-deep cores placed within PVC pipes to prevent the ingrowth of roots and mycorrhizal fungi) and "open" (i.e., 10-cm-diameter × 30-cm-deep ingrowth cores exposed to the bulk soil that could be colonized by roots and mycorrhizal fungi) cores were established in each plot. The ingrowth soil was collected from adjacent sites that received the same N amendments, sieved to remove coarse organic matter, and homogenized before filling the cores. The cores were established by filling the holes, tamping the soil to the approximate bulk density of the adjacent soil, and placing a PVC pin flag directly in the center to mark the core location. Thus, the open-core ingrowth soil was directly adjacent to the bulk soil (as opposed to being contained in nylon mesh bags used by Wallander et al. 2001). After either a 1- to 2-month ingrowth period to coincide with associated assessments of fine root dynamics, the cores were collected using a slightly smaller diameter corer (8 cm diameter × 30 cm deep) with the PVC pin flag serving as the center point (Hendricks et al. 2006a). The samples were composited by core type in each plot and placed in a cooler for transport to the laboratory.

Field samples were processed within 1 h of collection. In the laboratory, each sample was homogenized, and soil samples were collected for ergosterol concentration (5–7 g preserved in 5 ml of KOH methanol, 0.8% KOH in high-pressure liquid chromatography [HPLC] grade methanol, stored at −20°C; Gessner and Newell 2002) and moisture (5–7 g dried at 70°C to a constant mass; Jones 1984) analysis to express ergosterol concentrations on a dry-mass basis. After subsampling for ergosterol and soil moisture, the remaining soil for each open core sample was wet sieved (#10 mesh) to collect fine root samples to assess ectomycorrhizal fungal production in Hartig nets and mantles. Hartig net and mantle production were assessed jointly by extracting ergosterol from the fine root tissues that colonized the open ingrowth cores during the sample period. Isolated fine root tissues were rinsed with cold water, blotted dry, and preserved (5 ml KOH methanol, 0.8% KOH in HPLC grade methanol, stored at −20°C) for ergosterol analysis.

Ergosterol in the preserved soil and fine root samples was extracted in alcoholic KOH (0.8% KOH in HPLC grade methanol, total extraction volume of 10 ml) for 30 min at 80°C in tightly capped thick-walled digestion tubes. The resultant crude extract was cleaned by solid phase extraction (Gessner and Schmitt 1996), and ergosterol was purified and quantified by HPLC. A LichroSpher

100 RP-18 column (0.46×25 cm) maintained in a Shimadzu column oven (CTO-10AS) at 40°C and connected to a Shimadzu autosampler (SIL-10AD) and Shimadzu liquid chromatograph system (Pumps LC-10AT, Controller SCL-10A) was used for separation and analysis. The mobile phase was HPLC grade methanol at a flow rate of 1.5 ml min^{-1}. Ergosterol was detected at 282 nm using a Shimadzu (SPD-10A) UV/VIS detector (retention time = approx. 8 min) and was identified and quantified based on comparison with ergosterol standards (Fluka Chemical). Laboratory trials using this extraction procedure on autoclaved soil samples spiked with 25 μg of ergosterol yielded a 71.3±2.6% recovery of ergosterol. Consequently, estimates for the field samples were adjusted for unrecovered ergosterol. Ectomycorrhizal extramatrical mycelial production was calculated as the difference in ergosterol accrual between the paired open and closed cores.

Fungal sporocarp production was assessed in coordination with extramatrical mycelia, Hartig net, and mantle production assessments. At the end of each sample period, sporocarps were collected from five 1×1 m subplots in each of the 32 study plots. The sporocarps were dried at 70°C to a constant mass, weighed, and identified. Sporocarps that could not be conclusively identified visually were grouped based on similarity in appearance. Representative samples for each identified species and unknown group were homogenized using a Wig-L-Bug Model 6 Amalgamator (Reflex Analytical Corporation, Ridgewood, NJ). A 2- to 4-mg subsample of each sample was analyzed for ^{13}C and ^{15}N natural abundance at the U.C. Davis Stable Isotope Facility to potentially assess the trophic status (i.e., mycorrhizal vs saprotrophic) based on the significant difference in ^{13}C and ^{15}N natural abundance concentrations between mycorrhizal and saprotrophic species (Taylor et al. 1997; Gebauer and Taylor 1999; Hobbie et al. 1999; Högberg et al. 1999; Kohzu et al. 1999). In addition, a 3- to 5-g subsample was ashed (500°C for 4 h; Jones 1984) to express sporocarp masses on an ash-free, dry-mass basis.

The estimates of extramatrical mycelia, Hartig net and mantle, and ectomycorrhizal sporocarp production were summed for each sample interval. To express the production estimates in the same units (g/m^2), ergosterol production estimates associated with the extramatrical mycelia and root tips (i.e., Hartig nets and mantles) were converted to fungal biomass values using a conversion factor of 5 μg ergosterol per milligram fungal biomass based on the average of published estimates for ectomycorrhizal species (5.04±3.80 μg ergosterol per milligram fungal biomass; n= 36 cited in Salmanowicz and Nylund 1988; Martin et al. 1990; Antibus and Sinsabaugh 1993; Sung et al. 1995; Wallander et al. 1997; Colpaert et al. 1999). Fungal biomass estimates expressed in milligrams per gram of soil were then converted to a ground surface area basis (to a 30-cm depth) using a soil bulk density value of 1.15 g/cm^3. In turn, the extramatrical mycelia, Hartig net and mantle, and ectomycorrhizal sporocarp production estimates were summed across the sample intervals to yield an annual production estimate.

Edaphic resource availability assessments

Nitrogen mineralization was measured during the annual assessment using a closed core incubation technique (Raison et al. 1987; Blair 1997). Ten soil cores (2 cm diameter) were collected from the 0–10 cm horizon in each plot at the beginning of an incubation cycle, sieved (#10 mesh) in the laboratory, and subsampled for estimation of initial pools of inorganic N $\left(NH_4^+ \text{ and } NO_3^-\right)$ and moisture content. In addition, five subsamples were taken from each sample, placed in gas-impermeable PVC pipes (2 cm diameter) that were capped and buried (to 10 cm depth) in their original plot within a 24-h period. After an incubation period ranging from 28 to 35 days, samples were retrieved, composited by plot, and subsampled for inorganic N and moisture analyses.

Inorganic N in the initial and incubated soil samples was extracted with 2 M KCl (10 g:25 ml) by vigorous agitation on a mechanical shaker for 15 min, followed by centrifugation (2,500 rpm) for an additional 15 min. The supernatant for each sample was then carefully drawn off and analyzed colorimetrically for NH_4^+ and NO_3^- concentrations using a Lachat Flow Injection Analyzer (Kenney and Nelson 1982; Lachat Instruments 1992, 1997). Net ammonification and nitrification were then calculated by subtracting the initial concentrations from the final pools of extractable $NH_4^+ - N$ and $NO_3^- - N$, respectively. Net mineralization was calculated as the sum of net ammonification and nitrification during the incubation period expressed on a soil dry mass basis.

Soil moisture availability was assessed for each sample interval as well. In association with the N mineralization assessments, gravimetric soil moisture (g/g) was measured at the beginning and end of each sample interval. In addition, precipitation during the sample interval was measured at a weather station located approximately 2 km from the longleaf pine plantation.

Data analysis

Statistical analyses were conducted using mixed-models analysis of variance and regression analyses in SAS® (SAS Institute, Cary, NC). All variables were normally distributed, so data transformations were not necessary. Mixed-models analysis of variance was used to test for main and interaction effects of scorching and fertilization on ectomycorrhizal production through time (Littell et al. 1996).

Where significant interactions were present, contrasts were performed to detect specific differences. In addition, simple and multiple regression analyses were used to assess relationships between mycorrhizal fungi production and edaphic resource availability indices.

Results

Production rates

Ectomycorrhizal production including extramatrical mycelia, Hartig nets and mantles, and sporocarps was estimated to be 49 g m^{-2} $year^{-1}$ in the control plots of the longleaf pine plantation (Fig. 1). Extramatrical mycelia comprised 95% of the total annual production estimate. This can be considered a conservative production estimate, as extramatrical mycelial turnover may have occurred during the sample intervals. In contrast to extramatrical mycelia, the Hartig nets and mantles on root tips and mycorrhizal sporocarps constituted only 4.5 and 0.5%, respectively, of the total annual production (Fig. 1).

Estimates of mycorrhizal sporocarp production were also probably conservative because of the difficulty of positively identifying the trophic status of some specimens. Based on visual identification, 84% (by mass) of the sporocarps were confirmed to be ectomycorrhizal, belonging to the genera *Amanita*, *Cantharellus*, *Cortinarius*, *Geastrum*, *Laccaria*, *Pisolithus*, *Rhizopogon*, *Scleroderma*, *Thelephora*, and *Tricholoma*. Three percent of the sporocarps were confirmed to be saprophytic, belonging to the genera *Agaricus*, *Clathrus*, *Peziza*, *Polyporus*, and *Ramaria*, and 13% could not be conclusively categorized by trophic status. Natural abundance analyses of ^{13}C and ^{15}N also failed to conclusively reveal the trophic status of the "unknown" sporocarp specimen (data not shown). Consequently, the sporocarp masses used in the production estimate were limited to those conclusively identified as mycorrhizal. Although some mycorrhizal sporocarps were likely omitted from the production estimate, the error attributed to this source was considered small because: (1) the unknown specimens as a whole represented only a small percentage of the total sporocarp mass (13%), and (2) the sporocarps as a whole represented only a small percentage of the total ectomycorrhizal production estimate (<1%).

Temporal variation

Ectomycorrhizal production rates did not vary significantly (p=0.1366) during the annual assessment (Fig. 2). Although the differences were not significant, extramatrical mycelia did exhibit maximum production rates during the late June–early August sampling interval and minimum rates during the mid December–mid February sampling interval. The annual minimum rate of extramatrical mycelial production

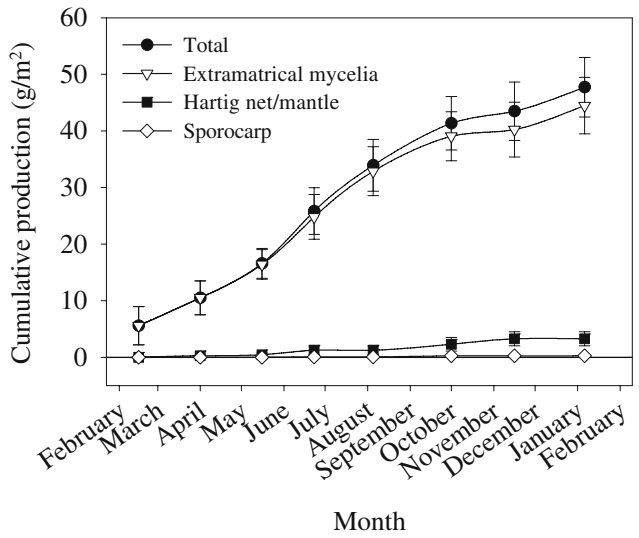

Fig. 1 Cumulative mycorrhizal fungi production in the control plots over the course of the annual assessment. Values represent means + SD for the sample interval

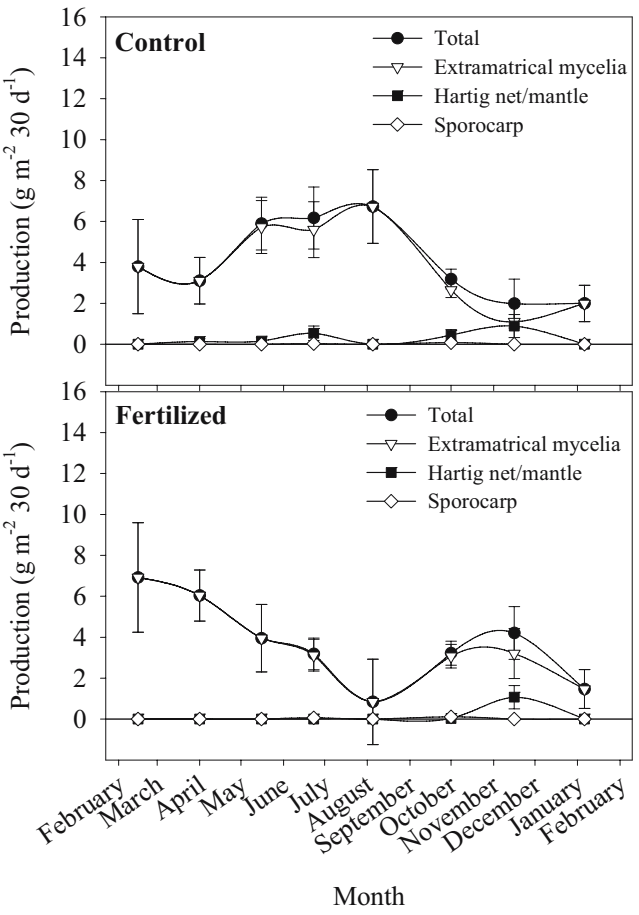

Fig. 2 Temporal pattern of mycorrhizal fungi production standardized to 30-day production intervals in the control and fertilized treatment plots

coincided with the maximum period of Hartig net and mantle production on fine roots. Mycorrhizal sporocarp production was consistently low throughout the study period.

Carbon source and sink controls

The mixed-models analysis of variance test for fixed effects did not reveal significant differences in total mycorrhizal fungi production because of scorching (p=0.9374; 47±4 vs 46±7 g m^{-2} $year^{-1}$ for scorched and nonscorched treatments, respectively) or fertilization (p=0.7865; 45±6 vs 48±4 g m^{-2} $year^{-1}$ for fertilized and nonfertilized treatments which exhibited mineralization rates of 2.0±0.2 and 0.9±0.1 g m^{-2} $year^{-1}$, respectively). However, there was a significant fertilization × time interaction effect (p=0.0414). Subsequent contrast analyses revealed that fertilization had a significant effect on ectomycorrhizal production during the fifth sampling interval (early August–mid September), which followed months of relatively large N additions in fertilized treatment plots (Fig. 2).

A linear regression analysis indicated that the relationship between N availability and total ectomycorrhizal fungi production was not significant (p=0.2210). However, a multiple regression analysis using both N availability and precipitation during the interval as the independent variables revealed a significant relationship (p=0.0292; r^2=0.42) with total ectomycorrhizal production during each sample interval (Fig. 3). The multiple regression equation (y=4.48−4.60[nitrogen availability] + 0.13[precipitation]) indicates that nitrogen had a negative impact, whereas precipitation had a positive impact on ectomycorrhizal production. Comparison of the absolute values of the standardized parameter estimates for N (−0.22) and precipitation (0.57) indicated that precipitation had a greater effect than nitrogen availability on total mycorrhizal fungi production.

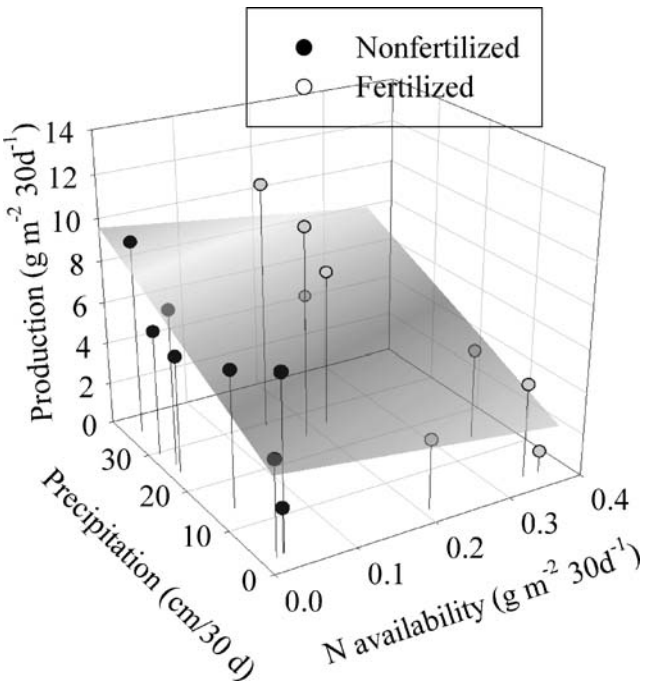

Fig. 3 Multiple regression relationship depicting the interactive effects of nitrogen availability and precipitation on mycorrhizal fungi production

Discussion

Mycorrhizal production dynamics are currently among the most poorly understood aspects of forest ecosystem ecology primarily because of the long-standing inability to assess the patterns and controls of extramatrical mycelia production in field settings (Treseder and Allen 2000; Wallander et al. 2001, 2004; Högberg and Högberg 2002; Hobbie 2006). The results of this study may provide valuable insight for several reasons. First, this study employed a modified version of the Wallander et al. (2001) ingrowth core approach (i.e., native soil matrix) to obtain more field-realistic estimates of extramatrical mycelia production, in association with assessments of Hartig net, mantle, and sporocarp production. Second, these production assessments were conducted over the course of 1 year in an established (i.e., 22-year-old) longleaf pine forest. Lastly, manipulations of C source strength and C sink strength to assess the ecophysiological controls on ectomycorrhizal production in the plantation were conducted in a manner that simulated natural disturbances and processes at the ecosystem scale.

Production rates

Ectomycorrhizal fungi production was estimated to be 49 g m^{-2} $year^{-1}$ in the longleaf pine plantation (Fig. 1). Hartig net, mantle, and sporocarps cumulatively constituted only 5% of the total production estimate, whereas extramatrical mycelia constituted 95% of the total (Fig. 1). Although few field-based ecosystem studies have assessed the proportional allocation of ectomycorrhizal production to extramatrical mycelia (Hobbie 2006), the estimate of 95% reported here is comparable to 60–85% reported by Colpaert et al. (1992) for a variety of ectomycorrhizal species in a laboratory-based study and approximately 80% reported by Wallander et al. (2001) for a field-based study in a Norway spruce forest ecosystem.

The annual ectomycorrhizal production estimate reported for this longleaf pine forest was approximately four times higher than the estimate reported by Wallander et al. (2001) for the Norway spruce forest (12.5 g m^{-2} $year^{-1}$ based on ergosterol analysis). In addition, crude estimates of the ratio of ectomycorrhizal fungi production to fine root production were higher in the longleaf pine forest (10.8%

based on an annual fine root production estimate of 448 g m^{-2} $year^{-1}$ derived for this site in 1999 using the minirhizotron approach; Carter et al. 2004) compared to the Norway spruce forest (3.5% based on an average fine root production estimate of 351 g m^{-2} $year^{-1}$ derived for this site from 1995 to 1996 using a root window observation approach; Stober et al. 2000). The differences in absolute and relative ectomycorrhizal production estimates between these studies may be attributed to the inherent differences in climate, edaphic resource availability, and net primary productivity between the two sites. However, the differences in ectomycorrhizal production estimates may also be attributed to the underlying ingrowth core methodology. Although both studies employed the ingrowth core approach, Wallander et al. (2001) used acid-washed sand as the ingrowth matrix, whereas we used native soil. In a previous study conducted in this same longleaf pine plantation site, Hendricks et al. (2006a) observed that ectomycorrhizal extramatrical mycelial production using native soil was 300% higher than corresponding production rates in acid-washed sand. This difference is consistent with the difference in ectomycorrhizal production observed between this and the Wallander et al. (2001) study.

Temporal patterns

Ectomycorrhizal production did not exhibit significant temporal variation during the annual assessment (Fig. 2). The lack of significant seasonal differences in the longleaf pine plantation contrasts with the patterns reported for boreal coniferous forests, where minimum and maximum ectomycorrhizal production rates generally occurred in the spring and fall, respectively (Wallander et al. 1997, 2001). Wallander et al. (2001) noted anecdotally that rates of ectomycorrhizal fungi production corresponded with fine root production rates reported by Stober et al. (2000), which were measured at the same study site but in a different year. The observation of relatively continuous ectomycorrhizal fungi production in the longleaf pine plantation is consistent with the fine root production patterns recently reported for this plantation (Carter et al. 2004) and other longleaf pine forests (West et al. 2004, Hendricks et al. 2006b), which typically have relatively mild winters and precipitation evenly distributed throughout the year (Mitchell et al. 1999; Kirkman et al. 2001). The relationship between fine root and ectomycorrhizal fungi production warrants further research.

Carbon source controls

Mycorrhizal fungi depend on C from the host plant for production and maintenance respiration. Although it is clear that the host may allocate a significant proportion of photosynthate to mycorrhizal fungi, the mechanisms that control this C flux to these fungal microsymbionts are poorly understood (Bidartondo et al. 2001; Högberg et al. 2001; Staddon et al. 2002, 2003).

Several studies have demonstrated that mycorrhizal fungi depend on recently fixed C, or current photosynthate, for production and maintenance activities (Söderström and Read 1987; Cairney et al. 1989; Cairney and Alexander 1992; Högberg et al. 2001; Hobbie et al. 2002; Högberg and Högberg 2002; Johnson et al. 2002; Treseder et al. 2004). Reductions in leaf area and current photosynthate production via defoliation, herbivory, or pruning have been shown to reduce C allocation to fine roots, and presumably their mycorrhizal fungal symbionts, in mature trees (Wargo et al. 1972; Parker and Patton 1975; Webb 1981; Eissenstat and Duncan 1992; Kielland et al. 1997; Ruess et al. 1998). Högberg et al. (2001) reported that stem girdling reduced soil CO_2 efflux rates by 37% within 5 days in Scots pine (*P. sylvestris*) forests. In addition, C isotope tracer assessments have indicated that mycorrhizal mycelia production and respiration are sustained using recently (e.g., ≤24 h) fixed C in a mature deciduous forest (Keel et al. 2006) and seedlings (Cairney et al. 1989; Cairney and Alexander 1992; Johnson et al. 2002; Wu et al. 2002; Staddon et al. 2003). Collectively, these studies suggest that disruption of current photosynthate production via anthropogenic or natural disturbances may have particularly adverse effects on mycorrhizal production.

In contrast, the finding that foliar scorching did not significantly reduce mycorrhizal production in this study suggests that stored C reserves may be used to support fine root and ectomycorrhizal fungi dynamics after disruption of current photosynthate production. It should be noted that stored C reserves and fluxes were not assessed in this study and that the C used to support mycorrhizal production on the roots of scorched trees may have been derived from other sources, most notably via mycorrhizal network connections to adjacent nonscorched trees (Southworth et al. 2005). However, it is unlikely that compensatory C supply via mycorrhizal networks was a significant C source in this system. Fine root and mycorrhizal dynamics were assessed in the interior subplot (15×15 m) of a large treatment plot (20×20 m), and C supply via a common mycorrhizal network likely decreases with distance from the original source (Southworth et al. 2005). In addition, the lack of a significant difference ($p=0.9374$) between mycorrhizal production in the scorched and nonscorched treatments was attributed to the similarity of the estimates (i.e., 47±4 vs 46±7 g m^{-2} $year^{-1}$ for scorched and nonscorched treatments, respectively) rather than an error associated with the estimates, and it is unlikely that mycorrhizal networks may totally compensate for the large

reduction in C supply strength of the scorched trees. In addition, the lack of a significant foliage-scorching treatment effect on ectomycorrhizal production is consistent with prior findings that foliage scorching did not significantly affect the production and mortality of fine roots (Carter et al. 2004) or the nonstructural carbohydrate concentration of fine roots (Guo et al. 2004), although this treatment did significantly reduce stored C reserves (e.g., coarse root C concentrations; Guo et al. 2004) in this longleaf pine plantation. This suggests that C reserves in coarse roots may be used to compensate for reductions in current photosynthate production and maintain the relatively high metabolic activity of fine roots and associated mycorrhizal fungi.

The apparent importance of stored C reserves for maintaining fine root and mycorrhizal fungi dynamics in longleaf pine forests is consistent with the findings of other studies conducted in systems subject to regular foliar disturbances. Kosola et al. (2001) observed that severe insect defoliation of *Eugeneii* hybrid poplars did not affect the nonstructural carbohydrate concentration or mortality rate of fine roots Edwards and Ross-Todd (1979) reported that stem girdling in a mixed deciduous forest did not significantly reduce fine root biomass or soil CO_2 efflux rates for up to 2 years after treatment initiation. Research by Langley et al. (2002), using ^{13}C tracer techniques, demonstrated that fine roots and associated ectomycorrhizal symbionts were maintained by stored C for more than 2.5 years after a prescribed burn in a scrub oak (*Quercus* spp.) ecosystem. In addition, foliage removal in grazing-tolerant grasses via burning or mowing also had no effect on fungal colonization or extramatrical mycelia development (Wallace 1987; Allen et al. 1989; Eom et al. 1999).

Although a direct linkage between current photosynthate production and fine root dynamics has been reported (Högberg et al. 2001; Johnson et al. 2002; Staddon et al. 2003; Treseder et al. 2004; Keel et al. 2006), other studies suggest that stored C may decouple the linkage between current photosynthate production and fine root dynamics in ecosystems subject to frequent foliar disturbances (e.g., fire, grazing, insect herbivory, etc.) (Eom et al. 1999; Kosola et al. 2001; Langley et al. 2002; Carter et al. 2004; Guo et al. 2004). Our results support the hypothesis recently proposed by Guo et al. (2004), which maintains that plant species may differ in their belowground C allocation relationships based on their natural history and resilience to disturbance.

Carbon sink controls

Plants use mycorrhizal fungi for nutrient acquisition, and thus the nutrient status of the host plant and soil has long been considered a primary factor influencing C allocation rates to mycorrhizal production (Allen 1991; Arnebrant 1994; Treseder and Allen 2000, 2002). Although it has been widely hypothesized that the host plant will allocate proportionately more C to associated mycorrhizal symbionts in nitrogen-poor environments (Read 1991; Smith and Read 1997; Wallenda and Kottke 1998; Treseder and Allen 2000, 2002), this hypothesis has not been rigorously tested in field settings because of the difficulty of measuring extramatrical mycelia production (Wallander et al. 2001; Högberg and Högberg 2002; Nilsson and Wallander 2003).

In this study, N fertilization reduced ectomycorrhizal production rates (Figs. 2 and 3). This pattern is consistent with recent observations reported by Nilsson and Wallander (2003), which indicated that N fertilization reduced extramatrical mycelial production by approximately 50% in a Norway spruce forest. In addition, in a recent meta-analysis of 31 studies, Treseder (2004) noted that mycorrhizal fungi colonization of fine root tips decreased 15% under N fertilization. Furthermore, the significant decline in ectomycorrhizal sporocarp production in forests subjected to atmospheric N deposition has been well documented (Wallenda and Kottke 1998; Cairney and Meharg 1999; Wallander et al. 1999; Treseder and Allen 2000; Lilleskov et al. 2002).

A multiple regression analysis indicated that N availability and precipitation together accounted for 42% of the variation in ectomycorrhizal production rates during the annual assessment (Fig. 3). Whereas N availability had a negative effect, precipitation had a positive effect on ectomycorrhizal production. The strong and contrasting effects of N and water availability on ectomycorrhizal production have important implications for C flow and nutrient cycling dynamic assessments in forests, as these resources may be altered significantly and independently by anthropogenic and natural disturbances (Norby and Jackson 2000; Treseder and Allen 2000; Aber and Melillo 2001; Staddon et al. 2002).

Implications for C flow and nutrient cycling assessments

This assessment of the rates and ecophysiological controls of ectomycorrhizal fungi production in the longleaf pine plantation support the widely speculated but poorly substantiated supposition that mycorrhizal fungi are a large and dynamic component of C flow and nutrient cycles in forest ecosystems (Hobbie 2006). Extramatrical mycelia, which have been traditionally ignored in field assessments, constituted the vast majority of total mycorrhizal fungi production. In addition, the manipulation of C source and sink strengths elucidated the importance of stored C, N, and water availability in the regulation of ectomycorrhizal production. Ecosystem-scale studies that assess the linkage between C assimilation and belowground allocation with an

emphasis on extramatrical mycelial production, respiration, mortality, and decomposition may provide valuable insight into the role of ectomycorrhizal fungi in C flow and nutrient cycle dynamics and are thus critical in assessing and predicting the responses of forest ecosystems to anthropogenic and natural disturbances.

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