Resilience of mycorrhizal fungi on defoliated and fertilized hybrid poplars

K.R. Kosola, D.M. Durall, G.P. Robertson, D.I. Dickmann, D. Parry, C.A. Russell, and E.A. Paul

Abstract: We examined the effects of fertilization and gypsy moth defoliation of hybrid poplar (*Populus* ×*canadensis* Moench 'Eugenei') on ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungal colonization, ECM richness, and ECM composition in the summers of 1997 and 1998. The factorial experiment included two levels of defoliation (defoliated and control) and fertilization (100 kg N·ha^{-1} and control). Gypsy moth (*Lymantria dispar* L.) populations were manipulated to obtain defoliation in the summer of 1996, 1997, and 1998; fertilization subplots were fertilized with NH_4NO_3 (100 kg N·ha^{-1}) in the spring of these years. There were no significant effects of defoliation on ECM or AM colonization in either year; there was a significant ($p \leq 0.05$) decline in AM colonization in fertilized plots in 1997 and a significant interaction between defoliation and fertilization effects on ECM colonization in 1997. In the nondefoliated plots, ECM fungal colonization increased, whereas AM colonization decreased because of fertilization. In the defoliated plots, neither ECM nor AM colonization was affected by fertilization. ECM community composition and richness were unchanged by any treatment. The small and transient effects of defoliation and fertilization on poplar AMs and ECMs demonstrate the tolerance of these early-successional trees to defoliation and their ability to rapidly use high levels of available nitrogen.

Key words: Populus, nitrogen, ectomycorrhizas, arbuscular mycorrhizas, ectomycorrhizas, *Lymantria dispar* (gypsy moth), defoliation.

Résumé : Au cours des étés de 1997 et 1998, les auteurs ont examiné les effets de la fertilisation et de la défoliation d'un peuplier hybride (*Populus* ×*canadensis* Moench 'Eugeni') sur la colonisation par les champignons ectomycorhiziens (ECM) et arbusculaires (AM), ainsi que sur la richesse en ECM et la composition des communautés ECM. L'expérience factorielle comportait deux degrés de défoliation (défolié et témoin) et de fertilisation (100g N·ha^{-1} et témoin). Ils ont également manipulé les populations de la lymantride spongieuse (*Lymantria dispar* L.), afin d'obtenir des défoliations, au cours des étés de 1996, 1997 et 1998; des sous-parcelles de fertilisation ont reçu 100 kg N·ha^{-1} au cours des printemps de ces années. Suite à la défoliation, aucun effet significatif n'a été observé, au cours de ces deux années, sur la colonisation ECM et AM; on a observé une diminution significative ($p \leq 0.05$) de la AM dans les parcelles fertilisées en 1997, et une interaction significative entre les effets de la défoliation et de la fertilisation sur la colonisation ECM, en 1997. Dans les parcelles non défoliées, la colonisation par les ECM a augmenté, alors que celles des AM a diminué, suite à la fertilisation. Dans les parcelles défoliées, ni la colonisation ECM, ni la colonisation AM ont été affectées par la fertilisation. La composition de la communauté ECM et sa richesse n'ont été affectées par aucun des traitements. Les effets faibles et transitoires de la défoliation et de la fertilisation sur les ECM et les AM du peuplier, démontrent la tolérance de ces arbres de début de succession à la défoliation ainsi que leur aptitude à utiliser rapidement un taux élevé d'azote disponible.

Mots clés : Populus, azote, ectomycorhizes, mycorhizes arbusculaires, *Lymantria dispar* (lymantride spongieuse), défoliation.

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Introduction

Large-scale outbreaks of defoliating insects, such as *Lymantria dispar* L. (gypsy moth), are common in forests throughout the world (Schowalter et al. 1986). Gypsy moth was introduced in 1869 to North America and has since slowly expanded its range. The most common hosts for gypsy moth are *Quercus*, *Populus*, and *Larix* species, with *Populus* usually being preferred (Liebhold et al. 1995). Hybrid poplars are one of the fastest growing temperate trees and have been used in short-rotation plantations for fiber and as a source of fuel in developing countries (Ceulemans and Deraedt 1999; Dickmann 2001).

Mycorrhizal fungi provide essential nutrients to the growing poplar tree, but at the same time they may compete with the tree for carbon under periods of defoliation (Eom et al. 2001). Because mycorrhizal fungi rely on their host plants for most of their fixed carbon, we expect that defoliation would adversely affect fungal colonization levels. Substantial leaf removal by herbivores often leads to a decrease in arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungal colonization (Bethlenfalvay and Dakessian 1984; Bethlenfalvay et al. 1985; Gehring and Whitham 1994 (depending upon soil fertility); Gehring et al. 1997; Rossow et al. 1997; Gange et al. 2002); however, there are frequent reports of either no effect or increased colonization in defoliated plants (Gehring and Whitham 1994; Markkola 1996; Hartley and Amos 1999; Saikkonen et al. 1999; Cullings et al. 2001; Eom et al. 2001; Gange et al. 2002).

Defoliation leads to the loss of leaf nitrogen; fertilization may moderate long-term declines in productivity caused by defoliation-induced nitrogen (N) deficiency (Bryant et al. 1991). Increased soil N availability, due to either fertilization or atmospheric N deposition (Raven and Yin 1998), may have a significant impact on forest mycorrhizal communities. ECM fungal colonization has been found to vary in response to N fertilization, with either no effect, a positive effect, or a decline in ECM colonization rates (Brandrud and Timmermann 1998; Neville 2001; Baum et al. 2002), depending on the plant species (Baum and Makeschin 2000). This variation may be due to varying responses by plants to different levels of soil N input (Wallenda and Kottke 1998; Peter et al. 2001) or different soil texture, soil nutrient content, and soil pH (Baum et al. 2002). Effects of N fertilization on colonization by AM fungi are also variable (Sylvia and Neal 1990; Vazquez et al. 2001).

Changes in ECM community composition may occur independently of changes in overall percent mycorrhizal colonization. N fertilization has been shown to change ECM community composition without affecting colonization or total species richness (Fransson et al. 2000; Peter et al. 2001). In addition, defoliation has been shown to change the ECM community on *Pinus sylvestris* seedlings without affecting the ratio of ECM root tips to total root tips (Saikkonen et al. 1999). The reduction of ECM species based on fruiting bodies was associated with N fertilization, although the number of ECM morphotypes observed in the soil was unchanged (Brandrud and Timmermann 1998). The impact of changes in ECM communities on forest composition is unknown, but it may lead to changes in forest ecosystem processes given that different ECM fungi are known to differ widely in their physiology (Rygiewicz et al. 2000).

Populus species are colonized by both AM and ECM fungi (Lodge and Wentworth 1990). Both types of mycorrhizal fungi can form a sink that competes for carbon with foliage developing after defoliation; they can also facilitate uptake of nutrients required for foliage production. Thus, the interaction between defoliation, AM and ECM colonization, and nutrient uptake by roots is expected to be complex (Eom et al. 2001), with potential effects on ecosystem carbon and nutrient cycling. To our knowledge, the interactive effects of defoliation and N fertilization on AM and ECM fungi have not been previously examined. In the present paper, we describe effects of both defoliation and N fertilization on the colonization of AM and ECM fungi and on ECM community composition of hybrid poplar (*Populus* ×*canadensis* Moench 'Eugenei') during a large-scale manipulated outbreak of gypsy moth.

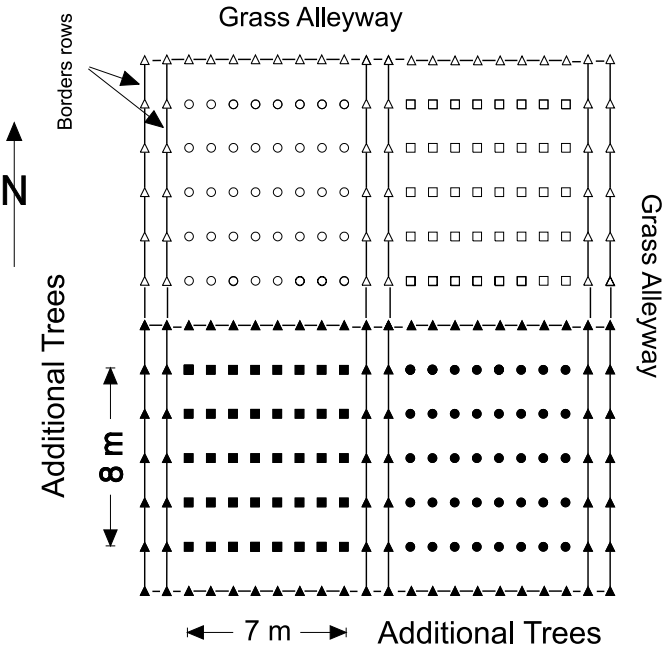
Materials and methods

Site description

In 1997 and 1998, mycorrhizal measurements were taken in four replicate blocks of hybrid poplars (*P.* ×*canadensis* 'Eugenei') on the Kellogg Biological Station Long Term Ecological Research site (KBS-LTER) in southwestern Michigan, USA. The LTER site had been planted with agronomic or forage crops for approximately 100 years before poplar hardwood cuttings were planted in May 1989. The experiment used a split-plot design, with two defoliation treatments as the main plots (defoliated and control), each split into a fertilized (100 kg $N \cdot ha^{-1}$ as NH_4NO_3) and unfertilized subplot (Fig. 1). Each subplot contained 40 experimental trees. The trees had an average diameter at breast height (DBH; 1.4 m) of 9.1 ± 0.1 cm in the spring of 1996. Two of the four replicate blocks were chosen randomly to have the defoliated plot on the exposed, northern portion of the block; the remaining two had the defoliated plot on the inner, southern portion of the block. Placement of the fertilized subplot on the exposed, eastern portion of each main plot was similarly randomly chosen for two of each of the defoliated and control plots, and then assigned to the inner, western portion of the other two plots of each defoliation treatment. This procedure was followed to maximize both randomization and dispersion of treatments (Hurlbert 1984). The plots are on a Kalamazoo sandy loam soil (Typic Hapludalf) and had soil phosphorus (P) levels of 80 μg $P \cdot g\ soil^{-1}$ in 1989 (Robertson et al. 1993).

Gypsy moth invaded the study area in the early 1990s. Initial populations were small, and consequently herbivory due to gypsy moth was minimal before the study started (S. Gage, personal communication). To create densities sufficient to cause significant defoliation, we introduced large numbers of gypsy moth egg masses into the plots beginning in 1996 while removing caterpillars from the undefoliated (control) plots (see Parry 2000 for details). First instar larvae were released into defoliation plots in 1996, followed by additional egg masses in 1997. In 1998, egg mass densities were high enough that supplementary egg masses were not added. Defoliation levels varied between years, with about

Fig. 1. Representative plot map. Closed symbols (●, ■, ▲) are undefoliated trees; open symbols (○, □, △) are defoliated trees; circles (●, ○) are unfertilized trees; squares (□, ■) are fertilized trees; triangles (▲, △) are border row trees. Plot size and tree spacing is to scale.



10% defoliation in 1996, 75%–100% in 1997, and 50%–100% in 1998 (Fig. 2; also see Parry 2000 and Kosola et al. 2001).

Tree measurements

Light transmission through the canopy in each of the replicate blocks was measured on overcast days with a 1 m long light (photosynthetically active radiation, PAR) sensor (Sunfleck Ceptometer, Decagon Devices, Pullman, Washington). Reference full-light measurements were taken outside of the plot before and after measuring canopy light transmission. Measurements were taken near the center of each subplot under healthy trees. Each reading was the average of 20 PAR measurements; 5 PAR measurements were taken while facing each of the cardinal directions, with the north–south measurements within rows and the east–west measurements between rows.

Tree DBH was measured at 1.4 m with a diameter tape (1996) or digital caliper (1997 and 1998; Forestry Suppliers, Jackson, Mississippi). The measurement points were marked on the bark with permanent marker, permitting consistent tape or caliper placement at each measurement date. Two caliper diameter measurements were taken for each tree (one north–south and one east–west) and averaged.

Mycorrhizal sampling and analysis

We sampled roots approximately 1 month after peak defoliation in both 1997 (29 July) and 1998 (28 July). Four 25 cm deep × 10 cm diameter soil cores were collected from randomly chosen locations in each subplot, split in half longitudinally, and stored on ice until processed. Each half core was weighed to provide an estimate of the proportion of total soil core volume in each subsample. Roots were extracted within 2 d from half of each core by hydropneumatic elutriation (Smucker et al. 1982) and stored in 24% (*v*/*v*) ethanol at 4 °C to preserve AM structures. The other half of each core was kept intact and stored in plastic bags at 4 °C for up to 6 months, until ECM root tips were extracted for analysis.

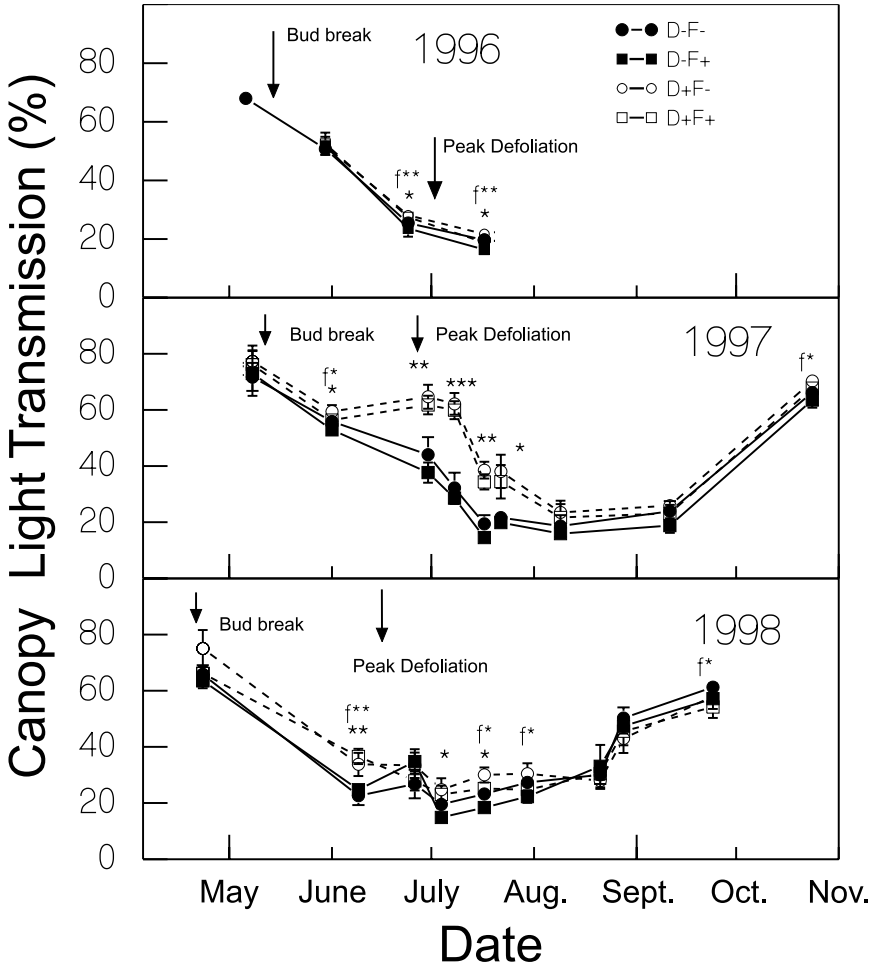
Roots stored in ethanol were cleared with KOH and stained with Chlorozol Black E (Jones et al. 1998). A line-intercept method (Giovanetti and Mosse 1980) was used under 400× magnification to determine the percentage of roots colonized by AM and ECM mycorrhizal fungi; therefore, all colonization data were calculated on a root length basis, with 500 line intercepts analyzed for each soil core. Root portions with a visible Hartig net or hyphal mantle were classified as colonized by ECM. Roots with vesicles, arbuscules, and (or) hyaline coenocytic hyphae running intercellularly through the cortex were classified as colonized by AM fungi (Jones et al. 1998). ECM subclasses were: Hartig net type 1, Hartig net type 2, Hartig net type 3, Hartig net + AM hyphae, Hartig net + arbuscules, and Hartig net + vesicles. Hartig net type 1 contained cells that were 1–2 µm in width with beaded hyphae surrounding the root cells. Hartig net type 2 contained cells that were 3–5 µm in width without beaded hyphae surrounding the root cells, whereas Hartig net type 3 contained cells that were 0.5–1 µm in width with beaded hyphae surrounding the root cells. The different classes of Hartig net represent morphotypes; they were clearly different in structure, but we do not know the taxonomic significance of these structural differences. Intersections of roots containing AM fungi were classified as hyphae, hyphae + vesicles, or hyphae + arbuscules. Pictures of these fungal structure combinations are located at http://lter.kbs.msu.edu/GypsyMoth/gypsymoth/summary.htm. Preliminary analysis showed that roots >0.5 mm were not significantly colonized by mycorrhizal fungi; therefore, only roots ≤0.5 mm were analyzed for colonization.

ECM root tips from the intact portions of the soil cores were extracted by gently washing on a 1-mm sieve. Morphological features of the ECM root tips were described and classified following the procedure of Goodman et al. (1996). The most common ECM fungal types (>10%) were further characterized by analysis of the restriction fragment length polymorphism (RFLP) pattern of the PCR-amplified product of the internal transcribed spacer (ITS) region of the fungal rDNA, as described in previous studies (Egger 1995; Baldwin and Egger 1996; Hagerman et al. 2001). The primers that were used to amplify the ITS region included ITS1 and NL6Bmun. The RFLPs were generated using *Mbo*I, *Hin*fI, and *Alu*I restriction enzymes. Samples from all four treatment combinations were analyzed in 1997. Analysis of ECM community composition was limited to samples from the undefoliated, unfertilized treatment in 1998.

Statistical analysis

Effects of defoliation and fertilization on mycorrhizal colonization by AM and ECM subclasses were analyzed as a split-plot design with the SAS MIXED procedure; blocks were treated as random effects, and fertilization and defoliation as fixed effects (Littell et al. 1996). Block averages for

Fig. 2. Canopy light transmission (percentage of total photosynthetically active radiation) at observation dates throughout the growing season in 1996, 1997, and 1998. Values are means ± SE; $n = 4$ for all data. Error bars not shown are smaller than the symbol. D, defoliation; F, fertilizer. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ for defoliation effects on light transmission. f*, $p < 0.05$ for fertilizer effects on light transmission.



total nonstructural carbohydrates and light transmission data at each date were analyzed as a split-plot design. Tree diameter growth and survivorship measurements were analyzed as a repeated measures, split-plot design, with measurements on individual trees nested within each subplot; the heterogeneous compound symmetric (CSH) option was used for the error structure of the repeated measure. All proportion data were arcsine square root transformed to improve normality after examination of residuals indicated significant departures from normality typical of proportion data (Sokal and Rohlf 1981). Planned Student's t test comparisons of all treatment combinations within each year and mycorrhizal class were carried out on least-squared means where significant interactions were detected (Fisher's protected T).

Results

Measurements of light transmission through the canopy in each plot gave an index of defoliation intensity, as light transmission is directly related to leaf area index (Buckley et al. 1999). The pattern and intensity of defoliation effects were different each year. Defoliation was moderate in 1996, uniformly severe in 1997, and varied among blocks from moderate to severe in 1998 (Fig. 2; Parry 2000). We found significant effects of defoliation on canopy light transmission at the time of peak defoliation in each year (Fig. 2). In 1997, peak defoliation at the end of June eliminated most of the canopy (Fig. 2). Leaf regrowth was rapid, but canopy densities in the defoliated plots were reduced until August (Fig. 2).

Fertilization increased tree diameter growth in 1996, 1997, and 1998 (Fig. 3, Table 1). DBH increment was significantly lower in defoliated plots in 1997 and 1998 (Fig. 3, Table 1). The significant interaction terms between measurement date and both fertilization and defoliation are due to within-season variation in growth response to these treatments. Analysis of simple effects (not shown) indicated that, after their first appearance, fertilizer and defoliation effects on early-season growth were maintained during the rest of the growing season (Fig. 3). The three-way interaction between defoliation, fertilization, and date in 1997 is due to a delay in the response of fertilized plots to defoliation; significant effects of defoliation were seen first in unfertilized plots (24 June) and later in fertilized plots (14 July). The

Fig. 3. Cumulative trunk DBH during the 1996, 1997, 1998, and 1999 growing seasons. Values are means ± SE; n = 4 for all data. Error bars not shown are smaller than the symbol. D, defoliation; F, fertilizer.

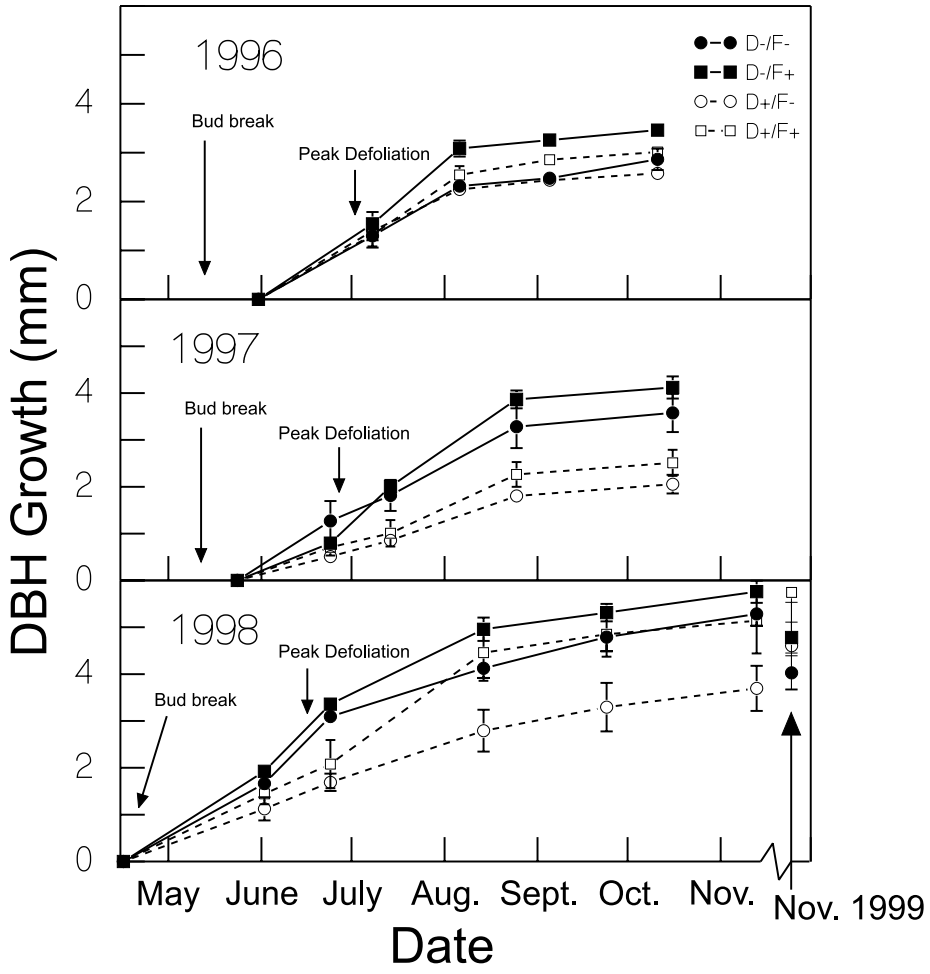


Table 1. Split-plot repeated measures ANOVA of diameter growth in 1996, 1997, 1998, and 1999.

		F values			
Source	df	1996	1997	1998	1999
Date	3	106.19****	166.69****	68.69****	
Defoliation	1	8.44	32.92****	20.17****	8.82*
Date × defoliation	3	0.99	25.89****	12.46****	
Fertilizer	1	6.96ns	10.54**	2.91ns	0.40
Date × fertilizer	3	4.21**	9.14****	1.48	
Defoliation × fertilizer	1	1.34	0.75	0.07	0.5607
Date × defoliation × fertilizer	3	0.62	4.41**	0.12	

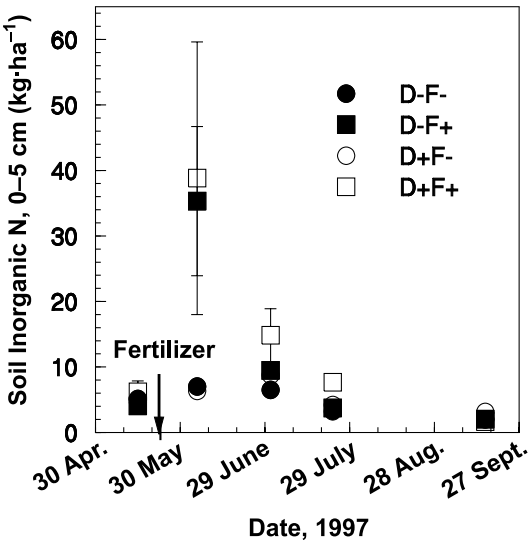
Note: Data for 1999 are from a single end-of-season measurement, so date effects for that year are not included in the analysis. ns, $p \le 0.1$; *, $p \le 0.05$; **, $p \le 0.01$; ***, $p \le 0.001$; ****, $p \le 0.0001$.

patterns of tree height growth were consistent with the patterns seen in DBH growth, with defoliation significantly decreasing growth in 1996 and 1998 (Kosola et al. 2001).

Fertilization transiently increased soil inorganic N levels; extractable N levels declined rapidly after fertilization (Fig. 4; Russell et al. 2004). Fertilization increased July leaf N levels in both defoliated and undefoliated treatments (defoliated fertilized plots, 2.61% ± 0.15%; undefoliated fertilized plots, 2.94% ± 0.21%; defoliated unfertilized plots, 2.38% ± 0.09%; undefoliated unfertilized plots, 2.71% ± 0.04%).

There was a small but significant ($p \le 0.01$) decline in AM hyphae + arbuscule colonization in fertilized blocks in 1997 (from 2.99% in control blocks to 2.44% in fertilized blocks) and a significant ($p < 0.05$) interaction between defoliation and fertilization effects on ECM colonization in 1997 (Ta-

Fig. 4. Extractable (2 mol/L KCl) inorganic N ($kg \cdot ha^{-1}$) in soil, 0–5 cm depth, 1997 growing season. Nitrogen (100 kg $N \cdot ha^{-1}$) applied as NH_4NO_3 on 23 May 1997.



ble 2). In the nondefoliated plots, ECM colonization increased with fertilization (from 14.99% to 18.01%), whereas AM hyphae + arbuscule colonization decreased with fertilization (from 16.22% to 14.80%). In the defoliated plots, ECM and AM hyphae + arbuscule colonization were unaffected by fertilization (Table 2). In 1998, a significant interaction between defoliation and fertilization effects on Hartig net type 1 colonization occurred (defoliated fertilized plots, 0.2%; undefoliated fertilized plots, 0.55%; defoliated unfertilized plots, 0.33%; undefoliated unfertilized plots, 0.33%; $p \leq 0.05$, Table 2), but this did not occur with Hartig net type 2 and type 3. Block effects were not significant.

Analysis of the ECM community showed no significant treatment effects on community composition or richness in 1997. In both 1997 and 1998, the most common type (>80% of ECM root tips in 1997 and 57% of ECM root tips in 1998) was morphologically and genetically indistinguishable from *Hebeloma crustuliniforme* (Table 3). There was a trend of an increase in *Tuber*-like morphotype by about 9% from 1997 to 1998, while *H. crustuliniforme* decreased by about 20% from 1997 to 1998 (Table 3). The RFLP patterns for these two morphotypes in 1997 and 1998 are listed in Table 4. The RFLP patterns for these two dominant types were consistent between sample cores. The RFLP pattern for *Tuber*-like morphotype number 4 sampled in 1997 differed slightly from that sampled in 1998. *Hebeloma crustuliniforme* has cream or white tips with a thin, cottony texture. The mantle is a felt or net prosenchyma with abundant clamp connections associated with the hyphae. The emanating hyphae are common and have abundant clamp connections. The *Tuber*-like morphotype is single or arranged irregularly in groups, has a smooth to short-spiny texture, and is light brown to grey; the mantle is relatively thick and is composed of an interlocking or noninterlocking irregular synenchyma. A key feature is the cystidia, which are 400 µm × 3 µm.

In 1997, 5 of the morphotypes were found in every block, 2 were found in two blocks, and the other 14 morphotypes

Table 2. Ectomycorrhizal (ECM, Hartig net) and arbuscular mycorrhizal (AM) colonization in each of the treatment combinations (defoliation (+, –), fertilization (+, –)) in 1997 and 1998, presented on a percent root length basis.

	1997				1998			
Type	(–), (–)	(–), (+)	(+), (–)	(+), (+)	(–), (–)	(–), (+)	(+), (–)	(+), (+)
AM hyphae	7.78 (1.10)	6.62 (1.19)	7.50 (1.57)	9.55 (1.42)	1.10 (0.46)	1.25 (0.09)	1.39 (0.32)	1.55 (0.23)
AM vesicles	1.29 (0.48)	1.40 (0.35)	1.24 (0.16)	1.84 (0.47)	0.36 (0.09)	0.34 (0.12)	0.44 (0.14)	0.50 (0.39)
AM hyphae + arbuscules	**3.26a (0.36)**	**2.54b (0.08)**	**2.71ab (0.50)**	**2.34b** (0.52)	1.80 (0.55)	1.41 (0.23)	1.63 (0.43)	0.79 (0.06)
AM total	12.33 (1.15)	10.55 (1.51)	11.45 (1.88)	13.73 (1.63)	3.26 (1.03)	3.00 (0.27)	3.45 (0.83)	2.84 (0.49)
ECM	**14.99b (1.60)**	**18.01a (0.34)**	**16.22ab (0.88)**	**14.80b (0.77)**	10.98 (0.78)	9.60 (1.94)	10.26 (1.18)	10.10 (1.40)
Hartig net type 1	2.09 (0.06)	3.09 (0.49)	2.60 (0.22)	2.76 (0.56)	**0.20a (0.08)**	**0.55b (0.12)**	**0.33ab (0.07)**	**0.30ab (0.06)**
Hartig net type 2	1.04 (0.23)	1.22 (0.19)	1.19 (0.42)	1.23 (0.21)	0.41 (0.09)	0.35 (0.13)	0.26 (0.08)	0.54 (0.21)
Hartig net type 3	0.08 (0.09)	0.03 (0.02)	0.03 (0.03)	0.00 (0.00)	0.16 (0.06)	0.09 (0.06)	0.09 (0.04)	0.19 (0.20)
Hartig net + AM hyphae	0.06 (0.05)	0.13 (0.09)	0.06 (0.07)	0.11 (0.08)	0.01 (0.01)	0	0	0
Hartig net + vesicles	0.05 (0.02)	0.05 (0.06)	0.01 (0.01)	0.11 (0.13)	0.01 (0.01)	0	0	0
Hartig net + arbuscules	0	0.01 (0.01)	0.03 (0.03)	0.04 (0.04)	0.05 (0.02)	0.01 (0.01)	0.03 (0.02)	0.01 (0.01)
Hartig net total	3.31 (0.17)	4.54 (0.56)	3.92 (0.38)	4.25 (0.82)	0.85 (0.11)	1.00 (0.18)	0.70 (0.05)	1.04 (0.19)
Other hyphae	7.69 (2.38)	7.01 (0.93)	6.97 (2.11)	9.66 (2.07)	4.31 (0.62)	4.38 (0.48)	2.83 (0.51)	5.04 (1.93)

Notes: Values are means (SE in parentheses), with $n = 4$. Pairwise Fisher's protected T tests were made among all treatments within each year for each observation class only where significant effects or interactions were detected by ANOVA. Values followed by the same letter are not significantly different at $p \leq 0.05$. Significant effects: ECM 1997, defoliation × fertilizer ($F_{[1,6]} = 7.66$, $p = 0.03$); AM hyphae + arbuscules 1997, fertilizer ($F_{[1,6]} = 15.42$, $p = 0.008$); Hartig net type 1 1998, defoliation × fertilizer ($F_{[1,6]} = 6.19$, $p = 0.05$).

Table 3. Percent relative abundance of the morphotypes formed by ectomycorrhizal fungi on roots of hybrid poplar in each of the treatment combinations (defoliation (+, –), fertilization (+, –)).

		1997				1998
Accession No.	Mycorrhiza type	(–), (–)	(–), (+)	(+), (–)	(+), (+)	(–), (–)
MSU001	*Hebeloma crustuliniforme* (Bull. ex Fr.)[a]	80.69 (11.40)	88.56 (5.35)	84.50 (5.48)	83.49 (5.87)	57.29 (12.59)
MSU004a	*Tuber*-like[a,b]	13.98 (3.20)	6.50 (4.52)	5.05 (5.54)	13.28 (5.14)	—
MSU004b	*Tuber*-like[a,b]	—	—	—	—	22.49 (7.48)
MSU804	Basidiomycete (dematiaceous)	—	—	—	—	9.77 (4.86)
MSU807	E-strain like	—	—	—	—	5.67 (3.78)
MSU802	Unknown	—	—	—	—	3.84 (4.43)
MSU009	Unknown	1.50 (1.73)	2.94 (3.40)	3.99 (2.77)	0.22 (0.15)	—
MSU013	Unknown	—	—	2.62 (3.03)	—	—
MSU003	Basidiomycete (dematiaceous)[a]	1.65 (0.83)	0.25 (0.29)	1.43 (0.74)	1.09 (0.74)	—
MSU011	*Thelephora*-like[a]	0.31 (0.36)	—	1.37 (1.48)	0.05 (0.05)	0.51 (0.59)
MSU008	Unknown	0.44 (0.50)	1.17 (1.35)	0.04 (0.04)	0.80 (0.33)	—
MSU806	Unknown	—	—	—	—	0.43 (0.49)
MSU015	Basidiomycete	0.36 (0.42)	—	—	—	—
MSU010	Basidiomycete	0.36 (0.42)	—	—	—	—
MSU017	Unknown	0.22 (0.25)	—	—	—	—
MSU014	Unknown	0.20 (0.23)	—	—	—	—
MSU021	Unknown	0.16 (0.18)	—	—	—	—
MSU012	Basidiomycete	0.13 (0.15)	—	—	—	—
MSU016	Unknown	—	—	0.58 (0.39)	—	—
MSU018	Unknown	—	0.32 (0.37)	—	—	—
MSU019	Unknown	—	—	0.43 (0.49)	—	—
MSU002	Unknown	—	—	—	0.56 (0.65)	—
MSU020	Unknown	—	0.25 (0.29)	—	—	—
MSU005	Basidiomycete	—	—	—	0.06 (0.07)	—
MSU006	Unknown	—	—	—	0.40 (0.46)	—
MSU007	Unknown	—	—	—	0.06 (0.07)	—
Total species richness		11	7	9	10	7

Notes: Values are means (SE in parentheses), with $n = 4$. Missing morphotypes are noted by a dash in the cell.
[a]PCR-RFLP analysis was performed.
[b]*Tuber*-like morphotype 4*b* collected in 1998 has different RFLP pattern from *Tuber*-like 4*a* collected in 1997.

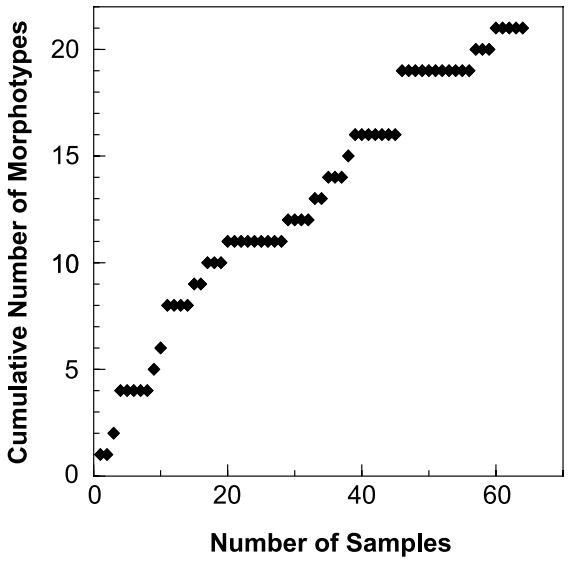
Table 4. Results of PCR amplification and restriction enzyme digest of fungal internal transcribed spacer sequences from ectomycorrhizal root tips sampled in 1997 and 1998.

				DNA fragment lengths (estimated bp)			
Year	Morphotype No.	Tentative ID	No. tips attempted / no. tips successful	Undigested	*Alu*I	*Hin*fI	*Mbo*I
1997	1	*Hebeloma crustuliniforme*[a]	5/5	1100	335, 250, 185, 175, 115	365, 265, 165, 150, 105	835, 270
1997	4*a*	*Tuber*-like[b]	10/6	925	605, 185, 115	275, 225x2, 135	375 × 2, 135, ~75
1998	1	*Hebeloma crustuliniforme*[a]	1	1100	335, 250, 185, 175, 115	365, 265, 165, 150, 105	835, 270
1998	4*b*	*Tuber*-like[b,c]	1	1050	425, 175, 150, 125	335 × 2, 235, 165, 150	435, 375, 240

Note: Each root tip analyzed was collected from a separate core. Where possible, replicate root tips were sampled from cores from different blocks and treatments.
[a]Matches sporocarps identified as *Hebeloma crustuliniforme*.
[b]*Tuber*-like ID based on morphological description.
[c]*Tuber*-like morphotype 4*b* collected in 1998 has different RFLP pattern from *Tuber*-like 4*a* collected in 1997.

were found in single blocks. Of the morphotypes occurring only in single blocks, 12 were observed in single cores, and 2 were observed in two cores. The morphotype community structure is highly skewed and dominated numerically by rare morphotypes. Out of the 21 morphotypes observed in 1997, 17 were observed in <1% of the ECM root tips. In

Fig. 5. Cumulative number of morphotypes observed with increased sampling effort (number of soil core samples).



1997, observations of new morphotypes continued to increase as sample numbers increased (Fig. 5). The smaller number of morphotypes (7 total) observed in 1998 is consistent with the reduction of sampling to control treatments: 64 cores were sampled in 1997, and 16 in 1998. Observation of roots in 1991 and 1992 showed only AM colonization (W.R. Horwath and E.A. Paul, unpublished data), indicating that the ECM population developed between 1993 and 1997.

Discussion

The *Populus* mycorrhizal system in the present study was both resilient and resistant to disturbance by fertilization and defoliation. This conclusion is supported by our observation that our treatment effects were consistently small and declined in the second year of the study despite annually repeated defoliation that was severe enough to cause a decrease in tree growth (Fig. 3, Table 1). One factor that may explain the resilience to disturbance of the mycorrhizal component in the *Populus* system is the relatively short duration of defoliation effects on the tree canopy. Gypsy moths did not consume the newest leaves on a tree, leaving a pool of untouched small leaves that expanded quickly. This returned the canopy to nearly full size within about 2 weeks (Fig. 2), likely resulting in a quick resumption of carbon allocation to roots. Other data from the KBS-LTER site also showed little response of poplar root carbon and N metabolism to moderate defoliation by gypsy moth and only a transient response to severe defoliation (Kosola et al. 2001). This rapid recovery of AM and ECM mycorrhizas from complete defoliation and resistance to moderate defoliation is consistent with the findings of Saikkonen et al. (1999) and Markkola (1996), where defoliation had no effect on *Pinus* mycorrhizas. In contrast, ECM colonization declined in browsed taiga trees (Rossow et al. 1997), and AM colonization increased in grazed tallgrass prairie (Eom et al. 2001). Browsing and defoliation have quantitatively different effects on tree physiology. Browsing leads to the combined loss of carbohydrate stores, leaf meristems, and photosynthetic area (Bryant et al. 1991), and so is likely to impose more extreme carbon limitations on the plant than the temporary loss of leaf area due to defoliation. Grazing can be prolonged throughout the growing season, unlike the 1-month period of defoliation by gypsy moth.

N fertilization at rates similar to those we used has previously been shown to have negative effects (Baum et al. 2002), positive effects (Baum et al. 2002), or no effect (Brandrud and Timmermann 1998) on mycorrhizal colonization. Inorganic N levels in the soil declined rapidly after fertilization (Fig. 4), although there were significant effects on growth (Fig. 3) and leaf N levels. The quality and magnitude of fertilization effects on mycorrhizal colonization can vary because of many factors including soil texture, soil N content, pH, and plant responses to soil N levels (Wallenda and Kottke 1998; Peter et al. 2001; Baum et al. 2002). In 1997, mycorrhizal response to N fertilization depended on both the defoliation treatment and mycorrhizal fungal type (ECM or AM). The interaction effect in 1998 was limited to Hartig net type 1 fungi, present only at very low levels (<1% colonization). These results demonstrate the complex nature of N fertilization effects on mycorrhizal colonization in poplar and other dual colonized plants such as willow (Baum et al. 2002).

The highly skewed community composition we observed, with a few dominant types and large numbers of rare types, is consistent with numerous studies of ECM communities (Bruns et al. 1998; Taylor 2002). For the sampling effort in 1997 (64 cores), the number of species is smaller than is found in gymnosperm communities with similar sampling effort (Horton and Bruns 2001). There is no clear evidence of a plateau in the plot of morphotypes observed versus sampling effort (Fig. 5), indicating that increased sampling effort would be likely to reveal additional rare morphotypes (Taylor 2002). Although these trees were planted on soil that had been planted to crops for about 100 years, diverse mycorrhizal inoculum from surrounding hardwood forest had successfully colonized the plantings.

Cullings et al. (2001) found ECM species-specific responses to 50% mechanical defoliation of lodgepole pine (*Pinus contorta*) in a mixed stand with Engelmann spruce (*Picea engelmannii*). Fungal species responses to the defoliation treatment varied from increased colonization, decreased colonization, or no change. In the present study, we did not observe a significant change in colonization by any ECM morphotypes after defoliation or fertilization. Other ECM species might have responded differently from *H. crustiliniforme*, but our power to detect changes in the more rare morphotypes was low.

Nitrate uptake capacity was 30% lower and ammonium uptake capacity 60% lower in defoliated plots than in nondefoliated plots in 1997, whereas there were no effects of defoliation on N uptake in 1998, when defoliation was not consistently severe (Kosola et al. 2001). Carbon allocation to late-season starch accumulation was significantly lower in defoliated plots in 1997, but not in 1998. Soluble sugar concentration in poplar roots was unaffected by defoliation or fertilization in any year. Root production, mortality, and biomass were not significantly affected by defoliation or fertilization in 1997 or 1998 (Kosola et al. 2001), reducing the chance of artifacts in colonization or richness estimates

caused by shifts in numbers of root tips (Taylor 2002; Wallenda and Kottke 1998). Root processes in unfertilized trees apparently vary in their sensitivity to defoliation, with N uptake and carbon allocation to starch being more sensitive than root growth, root mortality (Kosola et al. 2002), and AM and ECM mycorrhizal colonization (Table 2). Previous experiments with young poplar trees indicate that carbon allocation to roots is negligible following defoliation (Bassman and Dickmann 1985). The range of root process responses to defoliation suggests that there may be substantial variation in dependence of each process upon current photosynthate.

As early-successional trees, poplars have been subject to natural selection for tolerance to herbivory and an ability to rapidly use high levels of available N (Dickmann 2001). Consequently, poplar mycorrhizal symbionts are likely to be highly buffered from effects of defoliation and increased N availability by the physiological plasticity of the host tree. Similar resilience following defoliation may be seen among other early-successional trees.

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