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Dynamics of mycorrhizae during development of riparian forests along an unregulated river

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In this study, we explore two mycorrhizal groups during development of riparian soils along a freely-flowing river. We provide the first documentation of a shift in abundance between arbuscular mycorrhizae and ectomycorrhizae during floodplain succession. We used a chronosequence spanning 0–70 yr along a river in northwestern Montana, USA, to test the hypothesis that abundance of arbuscular mycorrhizal fungi (AMF) is greatest in early stages of soil development, and abundance of ectomycorrhizal fungi (ECMF) is greatest later in floodplain succession. We also measured the AMF-mediated process of formation of soil aggregates during site development. AMF colonization of the dominant tree (black cottonwood *Populus trichocarpa*) remained low (<5%), while AMF colonization of understory species was high (45–90%), across the chronosequence. Mycorrhizal inoculum potential (MIP) and hyphal length of AMF in soil peaked within the first 13 yr of succession and then declined. No single variable significantly correlated with AMF abundance, but AMF tended to decline as litter and soil organic matter increased. Density of ectomycorrhizal root tips in soil increased linearly throughout the chronosequence, and ectomycorrhizal colonization of cottonwood roots increased rapidly in early stages of succession. These patterns suggest that ECMF are not limited by dispersal, but rather influenced by abundance of host plants. Formation of water stable aggregates increased rapidly during the first third of the chronosequence, which was the period of greatest AMF abundance in the soil. The peak in AMF infectivity and hyphal length during early succession suggests that regular flooding and establishment of new sites promotes AMF abundance in this ecosystem. Regulation of rivers that eliminates creation of new sites may reduce contributions of AMF to riparian areas.

Globally, floodplains are some of the most threatened ecosystems (Tockner and Stanford 2002, Naiman et al. 2005). Although riparian areas often host high regional biodiversity, regulation of rivers changes fluvial dynamics that are required to maintain this diversity (Tockner and Stanford 2002, Naiman et al. 2005, Poole et al. 2006). High habitat diversity is maintained on floodplains through time as surfaces are recycled by the river through cut and fill alluviation (Ward et al. 2002). This process creates a shifting habitat mosaic of floodplain surfaces in different stages of plant succession (Stanford et al. 2005, Whited et al. 2007). Without regular flooding of different intensities, riparian vegetation may mature into relatively homogenous stands or be replaced by non-native species (Howe and Knopf 1991). For example, cottonwood trees (*Populus* spp.) dominate early-successional sites along many rivers in the northern hemisphere. Cottonwoods specialize in establishing on new surfaces created by seasonal floods (Karrenberg et al. 2002), and without floods these trees often senesce without replacement (Howe and Knopf 1991, Braatne et al. 1996, Poiani et al. 2001). As this displacement is documented for cottonwoods, the same may occur with other taxa, both above and below ground. A better understanding of the above- and belowground components of riparian areas during succession will be critical in preserving floodplain biodiversity and function (Naiman et al. 1993).

Mature floodplain soils are often nutrient rich and highly productive compared to surrounding upland soils because of constant nutrient inputs from headwater and lateral drainages (Gregory et al. 1991, Tockner and Stanford 2002). Soil development and diversity are important aspects of the shifting habitat mosaic, but they have not been widely studied in this context. Mycorrhizal fungi and other soil organisms affect development of soil as well as the plant community directly and through their effect on plant productivity (Rillig 2004, Rillig and Mummey 2006). Mycorrhizal associations are ecologically significant mutualisms between soil fungi and over 80% of all terrestrial vegetation (Smith and Read 1997). Mycorrhizal fungi often confer benefits to their plant hosts, such as increased access to immobile nutrients

greater tolerance to drought, and protection from pathogens (Smith and Read 1997). However, very few studies to date have examined the fungal component of developing floodplain soils (Jacobson 2004, Beauchamp et al. 2007).

During development of riparian forests, patches of vegetation within the habitat mosaic undergo succession. As the aboveground community changes in abundance and composition, so too may the soil community. In other temperate and boreal successional systems arbuscular mycorrhizal fungi (AMF) are the primary mycorrhizal associate in early succession, whereas in older soils the main associates are ectomycorrhizal fungi (ECMF) (Johnson et al. 1991, Boerner et al. 1996, Barni and Siniscalo 2000, Treseder et al. 2004). The mechanism of this shift is proposed to be related to soil nutrient status (Read 1991), but concurrent changes in other soil properties and plant community composition make it difficult to isolate a single causal agent. For instance, the effect could be driven by an increase in the abundance of conifer roots over successional time. Nevertheless, such a change in the dominant mycorrhizal association could have a number of ecosystem consequences, as these fungi differ in their functions. AMF affect phosphorus cycling, aid seedling establishment of many plant groups, help maintain plant diversity, and strongly contribute to soil stabilization and carbon storage through soil aggregate formation (Smith and Read 1997, van der Heijden et al. 1998, van der Heijden 2004, Rillig 2004, Rillig and Mummey 2006). Conversely, ECMF contribute to decomposition, organic nitrogen cycling, and conifer establishment (Smith and Read 1997, Read and Perez-Moreno 2003, Ashkannejhad and Horton 2006). If AMF abundance follows the same pattern during floodplain succession as has been shown in other studies of temperate succession, then river regulation that limits creation of young sites would be expected to affect AMF abundance, and thus plant diversity, soil stabilization, and soil carbon storage.

The Nyack floodplain at the southern boundary of Glacier National Park, Montana, USA, offers a model system to study mycorrhizae during floodplain development. It is one of the longest, freely flowing segments of river in the continental U.S., and it also has protected headwaters. This floodplain has a mosaic of habitat patches of known age since flooding deposited the foundation material, all within several kilometers of each other (Stanford et al. 2005, Whited et al. 2007). The main objective of this study was to test the hypothesis that AMF are most abundant in early successional soils and ECMF are most abundant in late successional soils. Additionally, we characterized changes in abiotic and biotic site variables through time that may affect AMF abundance. Lastly, we documented the change of a key AMF mediated process, soil stabilization, during floodplain development to understand if soil stabilization is related to AMF abundance in floodplain development. Results of this study will serve as a reference for studies of mycorrhizal dynamics along rivers with altered flow regimes and provide insight into soil processes that may aid in river restoration.

Methods

Site description

The Nyack floodplain is located in northwestern Montana (48°27′30″N, 113°50′W), on the Middle Fork of the Flathead River, a 5th order, free-flowing river with protected headwaters (catchment area = 2300 km^2). The Nyack floodplain is ca 2 km wide and 10 km in length and is comprised of active and abandoned channels, spring brooks, ponds and stands of regenerating and mature riparian vegetation. Actively scoured areas of the floodplain consist of gravel bars with shallow ponds, debris, and vegetation patches (Stanford et al. 2005).

This floodplain has high regional plant diversity, hosting over 200 plant species (Mouw 2001, Mouw and Alaback 2003). Common vegetation at our study sites (Table 1) is similar to other high latitude cottonwood-dominated riparian systems (Helm and Collins 1997). Following floods on Nyack, dense patches of cottonwood seedlings establish on top of freshly deposited sediment. Forbs and grasses that host AMF also recruit within the first couple of years. By ten years, cottonwoods establish a dense thicket with a grass and herbaceous understory. The earliest conifer seedlings occur between 10 and 15 yr, and are very sparse (Piotrowski unpubl.). By 28 yr post disturbance, cottonwood density has decreased, and a dense, primarily grass understory exists with occasional conifers. This structure eventually yields to a mixed cottonwood and conifer forest and diverse grass, herbaceous, and woody understory (Mouw 2001, Mouw and Alaback 2003). Thus,

Table 1. Common plant species along the Nyack chronosequence (adapted from Mouw 2001, Mouw and Alaback 2003) and their occurrence across site ages.

Plant types	Mycorrhizal associates of the plant family	Sites present
Herbaceous		
Agrostis gigantea	AMF	All sites
Arnica cordifolia	AMF	34, 50
Melilotus officinale	AMF	1, 4, 7, 10, 12, 34
Smilacina racemosa	AMF	34, 69
Centaurea maculosa	AMF	All sites
Verbascum thapsus	AMF	4, 7
Achillea millefolium	AMF	28, 34
Woody shrubs		
Rosa woodsii	AMF/ECMF	53, 69
Symphoricarpos albus	AMF	31, 37, 53, 69
Crataegus sp.	AMF/ECMF	50, 69
Cornus stolonifera	AMF/ECMF	13, 34, 53, 69
Rubus parviflorus	AMF/ECMF	66
Salix spp.	AMF/ECMF	16, 50, 69
Alnus tenuifolia	AMF/ECMF	34, 53, 69
Deciduous trees		
Amelanchier alnifolia	AMF/ECMF	53, 69
Populus trichocarpa	AMF/ECMF	All sites
Acer glabrum	AMF	34, 53, 69
Prunus virginiana	AMF/ECMF	69
Coniferous trees		
Abies spp.	ECMF	34, 50, 69
Picea spp.	ECMF	28, 34, 50, 69
Pseudotsuga menziesii	ECMF	34, 50, 69

both AMF and ECMF hosting plants are abundant at all sites.

The portion of the Nyack floodplain chronosequence we employ is composed of sites of 11 ages, ranging from 0 yr (freshly deposited sediment) to 69 yr (mature mixed forest). Aging of sites along the Nyack floodplain is based on the average age of cottonwood trees at each site. Because cottonwoods colonize sites shortly after disturbance and often recruit as even-aged stands, their age often reflects the time since disturbance (Everitt 1968). All sites on Nyack were initially aged in the summer of 2000 by coring cottonwoods (Harner and Stanford 2003).

Sample collection

We sampled along the Nyack chronosequence during October of 2003, October 2004, and June–August of 2005 at sites ranging from 1 to 69 yr old. We aged and sampled young sites (<5 yr post disturbance) each year as these sites may be lost to flooding yearly prohibiting return to all original young sites. We sampled from the same older sites (7–69) every year. While we collected materials over a three-year period, we present the age of the sites we returned to (7–69) as their age at the first collection within graphs and tables. During 2003 we were able to collect three sub-samples from three replicate one year sites, thus hyphal length, fine root colonization, litter, and herbaceous biomass measurements of this age represent nine sub-samples. Additionally, in 2005 we collected freshly deposited sediment from three sites, which we considered zero years old.

For soil analysis and arbuscular mycorrhizal measurements we collected ca 4 L of soil from the top 10 cm beneath the litter layer from three randomly selected locations (five during 2005) within each of the different aged sites. We collected cottonwood roots for percent ectomycorrhizal colonization determination in October 2004 from five random cottonwood trees within each aged site. For ECMF tip density measurements we collected whole soil samples (including soil and total roots) from three randomly selected areas per aged site using a corer (5 cm in diameter) to a depth of 10 cm. We did not collect soil from the 12 and 37 yr old site for the MIP bioassay because high water limited access to the site. We were able to access the sites later in summer to collect for ECMF tip density measurements later in the year.

Site and soil characterization

We measured abiotic characteristics of soil on three replicate samples from each site that we collected in 2003. We selected one subsample from each one-year-old site for analysis, thus the means of soil variables at one year is of three samples. Samples were analyzed at South Dakota Univ. soil testing laboratory for pH, Olsen phosphorus, potassium, nitrate, soil organic matter, and soil texture. Soil pH was analyzed in 1:1 soil:water (w/v). Soil organic matter was measured using the loss on ignition (LOI) method described in Combs and Nathan (1998).

We measured changes in the AMF-hosting herbaceous understory by clipping, drying, and weighing aboveground herbaceous material from three randomly selected 900 cm^2 plots per site. We used the same area to estimate litter accumulation at each site. We collected litter during a single sampling event rather than over a season; however, collection was after cottonwoods had lost the majority of their leaves and represents near maximum litter accumulation for a season. We dried litter and understory biomass for 2 d at 80°C, and weighed. We converted these values into grams understory biomass or litter per square meter. We unfortunately lost one litter replicate from the four-year-old site and one biomass replicate each from the 7, 13, and 15 yr sites, thus these site averages represent the mean of two samples.

AMF measurements

To determine how AMF change in abundance across the Nyack chronosequence, we assessed AMF colonization of random fine roots from the soil, AMF colonization of the cottonwoods, AMF potential (MIP) across the chronosequence, and AMF soil hyphal lengths. We collected fine cottonwood roots attached to three cottonwood trees at each site in August 2005. We collected fine roots from the soil by sieving the soil and picking out roots with forceps from the 2003 soil samples. We stained the community fine roots with trypan blue as described by Brundett (1994). We stained cottonwood roots the same way with the addition of a 5 min 20% bleaching step after roots were cleared with KOH. Arbuscular mycorrhizal colonization (including presence of hyphae, vesicles and arbuscules) was assessed at 200 × on a Nikon Eclipse E600 microscope by the gridline intersect method (McGonigle et al. 1990) at ca 50 randomly selected locations per slide.

Mycorrhizal inoculum potential is directly related to the abundance of infectious AMF propagules (spores, hyphae, infected root fragments) present in a soil (Johnson 1993). To determine AMF inoculum potential across the chronosequence, we modified the MIP method described by Boerner et al. (1996). Fresh field soil (100 g) was collected in July 2005 and transferred into 115 ml Cone-Tainers™ (Stuwe and Sons, Canby, OR). We used replicates from four random samples from each aged site in the bioassay. Each pot received 3 seeds of sudan grass *Sorghum sudanese* that were thinned to two plants per Cone-Tainer after germination. Sudan grass is routinely used for MIP measurements as it is a good host for AMF (Johnson 1993). We grew the plants under ambient greenhouse conditions for 30 d, and plants were watered with tap water as needed. We lost three plants during growth from the one year site, thus the MIP data from this age represents only one replicate. Roots were stained and AM colonization was estimated as described above.

We estimated soil abundance of AMF by measuring hyphal lengths in bulk soil. External hyphae were extracted from 4.0 g portions of soil and lengths were measured by a gridline intersect method at 200 × (Jakobsen et al. 1992, Rillig et al. 1999). We distinguished hyphae of non-AMF fungi from AMF by observing characters normally missing in the latter: melanization, clamp connections or regularly

septate hyphae, non-dichotomous branching (Rillig et al. 1999).

ECMF measurements

We estimated percent ectomycorrhizal colonization [(number of ectomycorrhizal root tips/total number of root tips assessed) × 100] by screening a gently rinsed sub-sample of cottonwood roots collected in October 2004 under a dissecting scope. We randomly screened 100 root tips for each of the five samples collected from each site. We considered any root tips with visible mantle development and morphology and color differing from the long, narrow, orange appearance of non-infected cottonwood roots to be colonized by ECMF.

We estimated ECMF abundance by collecting whole soil samples as described above in August 2005. Between 10 and 80 mL of homogenized whole soil was immersed in water over a 1 mm sieve to remove most of the soil and rinsed gently to avoid damaging the mycorrhizae. The content on the sieve was collected and examined under a dissecting scope. We counted the total number of ectomycorrhizal tips in each sample. We never assessed hyphal lengths of ECMF because ECMF cannot be distinguished from non-mycorrhizal fungal hyphae (e.g. saprobes and pathogens; Wallander et al. 2001).

Water stable aggregate measurements

We measured the percent of water stable aggregates of the 1–2 mm diameter size class (% WSA_{1-2mm}) as a measure of physical soil structure (Kemper and Rosenau 1986). We sieved air dried soils and collected the 1–2 mm fraction from three replicates within each aged site. We used 4 g of the fraction for the analysis and moistened replicate samples of soil aggregates by capillary action for 10 min before measuring stability. We measured water-stability of aggregates with a wet-sieving method using the apparatus and procedure described in Kemper and Rosenau (1986). We calculated percentage of water-stable aggregates (% WSA_{1-2mm}) using the mass of aggregated soil remaining after wet sieving (5 min) and the total mass of aggregates at the beginning, correcting the initial and final weights of aggregates for the weight of coarse particles (>0.25 mm) included in the soil samples.

Data analysis

We analyzed change of soil properties, litter, and herbaceous biomass through time with Spearman' s rank correlation on the means from each site and site age using NCSS 2000 (NCSS, Kaysville, UT, USA). We used regression analysis, after testing that the assumptions of normality and homoscedascity were met, to determine how mycorrhizal variables and water stable aggregate formation change with time using only the means (not individual samples, which would constitute pseudo-replication) of response variables from each aged site with SigmaPlot 7.101 (SPSS Chicago, IL). Changes in AMF, ECMF, and aggregate formation across the chronosequence followed a distinctly nonlinear pattern, and because we had no a priori ecological basis on which to select a model for over this period of time we chose the model that best described the data. We verified the appropriateness of the nonlinear models by calculating Akaike' s information criterion (AIC) values for the model compared to a linear model. All nonlinear models selected had a lower AIC than linear models. To test if any soil or site variables, including percent water stable aggregates, were correlated with AMF hyphal length we conducted Spearman' s rank correlations using NCSS 2000.

Results

Abiotic and biotic changes through time

Changes in abiotic variables along the chronosequence are presented in Table 2. While soil pH did not change dramatically across the chronosequence, it was negatively correlated with site age ($p < 0.05$). Additionally, nitrate was negatively correlated with site age ($p < 0.05$), whereas soil phosphorus and potassium were positively correlated with age ($p < 0.05$). Soil organic matter correlated positively with site age, displaying close to a ten-fold increase between 4 and 31 yr ($p < 0.05$). Percent sand was negatively correlated with age, while percent silt and clay were both positively correlated with age ($p < 0.05$). Changes in surface litter and understory biomass are presented in Table 3. Herbaceous understory biomass and litter were both positively correlated with site age ($p < 0.05$).

Table 2. Abiotic soil parameters of aged sites along the Nyack chronosequence (mean ± standard error) and Spearman' s correlation values of the variables correlated with site age. ("*" indicates significance at $p < 0.05$).

Site age	pH	NO_3^- mg kg^{-1}	% Sand	% Silt	% Clay	% OM	K mg kg^{-1}	P mg kg^{-1}
1	8.0 (0.0)	5.0 (2.6)	69.3 (2.19)	16.7 (1.8)	14.0 (0.6)	0.7 (0.8)	39.0 (3.5)	2.7 (0.3)
4	8.1 (0.0)	1.5 (0.3)	79.7 (1.45)	8.3 (1.2)	12.3 (0.3)	0.4 (0.0)	37.0 (1.5)	2.0 (0.0)
7	8.1 (0.1)	1.8 (0.6)	78.0 (5.77)	9.7 (4.4)	13.0 (1.5)	0.6 (0.2)	59.0 (8.7)	2.0 (0.0)
13	8.1 (0.0)	1.0 (0.5)	71.0 (2.08)	16.7 (1.3)	12.3 (0.9)	0.7 (0.0)	54.0 (6.0)	2.0 (0.0)
15	8.1 (0.0)	1.7 (0.2)	71.3 (0.67)	16.7 (0.7)	12.0 (0.0)	0.7 (0.0)	52.0 (5.7)	1.7 (0.3)
19	7.8 (0.0)	1.0 (0.0)	51.7 (3.18)	31.3 (2.7)	17.3 (0.9)	1.7 (0.2)	76.7 (5.5)	3.3 (0.3)
31	7.7 (0.0)	0.8 (0.2)	26.7 (2.67)	54.7 (8.2)	19.3 (6.2)	3.7 (0.2)	89.7 (5.4)	3.7 (0.3)
37	7.6 (0.0)	1.2 (0.2)	42.7 (5.21)	38.7 (5.2)	19.3 (0.9)	2.7 (0.4)	98.3 (7.1)	4.0 (0.0)
53	7.8 (0.0)	1.0 (0.0)	38.7 (3.71)	42.0 (3.1)	19.3 (0.7)	2.0 (0.4)	90.3 (2.7)	3.0 (0.0)
69	7.7 (0.1)	0.8 (0.2)	50.3 (8.09)	34.0 (7.2)	15.7 (0.9)	2.4 (0.1)	96.0 (6.7)	3.3 (0.3)
r_s	0.75*	−0.76*	−0.76*	0.82*	0.65*	0.82*	0.90*	0.63*

Table 3. Biotic parameters of aged sites along the Nyack chronosequence (mean ± standard error) and Spearman's correlation values of the variables correlated with site age. ("*" indicates significance at $p < 0.05$).

Site age	Herbaceous (understory) biomass (gm^{-2})	Litter biomass (gm^{-2})
1	15 (3)	0 (0)
4	39 (5)	24 (9)
7	48 (16)	110 (37)
13	42 (1)	479 (61)
15	20 (7)	415 (114)
19	118 (18)	488 (98)
31	79 (22)	916 (101)
37	124 (10)	529 (89)
53	156 (26)	600 (137)
69	64 (18)	423 (24)
r_s	0.78*	0.76*

Changes of mycorrhizae across the chronosequence

AMF colonization of cottonwood roots was low across the entire chronosequence, averaging <2% and ranging from 0% at most sites to 4.4% at the youngest site (data not shown). Occasional vesicles were present, but very few arbuscules were visible in the cottonwood roots. Cottonwood roots also hosted non-AMF in roots. We observed regular septa and clamp connections in some hyphae, indicative of fungi other than AMF, when examined at 400×. AMF colonization of understory, non cottonwood fine roots displayed a peak early in site development (Fig. 1). AMF colonization of fine roots ranged between 45 to 90%, increasing rapidly early in site development (0–5 yr) then steadily declining to 30 yr post disturbance after which colonization increased slightly.

AMF inoculum potential (Fig. 2) and soil hyphal length of AMF (Fig. 3) changed significantly during succession, and both fit a lognormal 4-parameter nonlinear model (adj. $R^2 = 0.58$ and adj. $R^2 = 0.68$ respectively, $p < 0.05$, equation presented in figure legend), which describes a rapid increase to a peak followed by a decline phase. The peak in inoculum potential occurred earlier (9 yr post disturbance in 2005, presented as 7 yr in graph for consistency) than the peak hyphal lengths (13 yr post disturbance); however, hyphal lengths were near maximum by this age as well. We extracted AMF hyphal lengths from 2005 soil samples, and these had a similar trend, with a peak in hyphal lengths the same site age as inoculum potential (data not presented). No site variables measured were significantly correlated with AMF hyphal lengths across the chronosequence.

Ectomycorrhizal colonization and tip density in soil increased across the chronosequence. ECMF colonization of cottonwood roots increased rapidly early in site development (Fig. 4) and significantly fit a single rectangular two-parameter hyperbolic model (adj. $R^2 = 0.95$, $p < 0.05$, equation presented in figure legend), which describes a rapid increase to a stable level. The soil density of ectomycorrhizal roots tips increased linearly across the chronosequence (Fig. 5; adj. $R^2 = 0.98$, $p < 0.05$), with the greatest density at the oldest site.

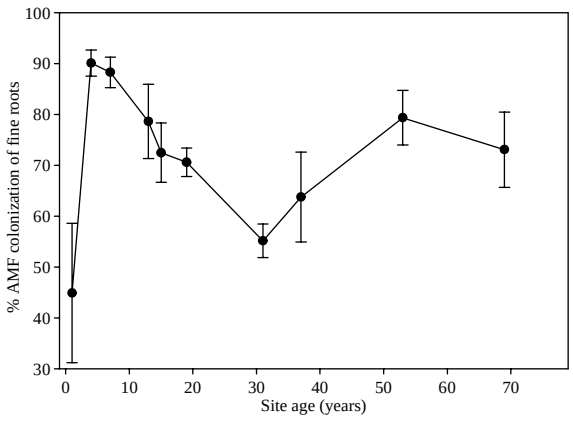

Fig. 1. AMF colonization of understory fine roots in October 2003 from bulk soil across the Nyack chronosequence (mean ± standard error).

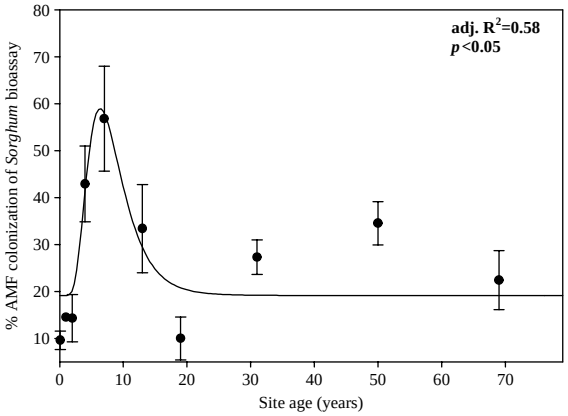

Fig. 2. Mycorrhizal inoculum potential across the chronosequence in July 2005 as measured by percent colonization of *Sorghum* bioassay fitted along the lognormal 4 parameter nonlinear model ($(y = y0 + a\hat{}[-0.5(\ln(x/x0)/b)^2])$, where $a = 39.83$ $b = 0.43$, $x0 = 6.36$, and $y0 = 19.14$ (mean ± standard error).

Changes in % WSA_{1-2mm}

Percent WSA_{1-2mm} increased (Fig. 6) during the first half of the chronosequence and significantly fit a single rectangular

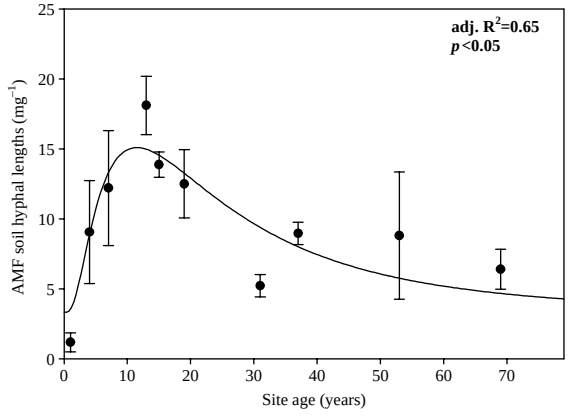

Fig. 3. Changes in AMF biomass as measured by soil hyphal lengths (m g^{-1} soil) across the Nyack chronosequence fitted along the lognormal 4 parameter nonlinear model, where $a = 11.7$, $b = 0.86$, $x0 = 11.5$, $y0 = 3.31$ (mean ± standard error).

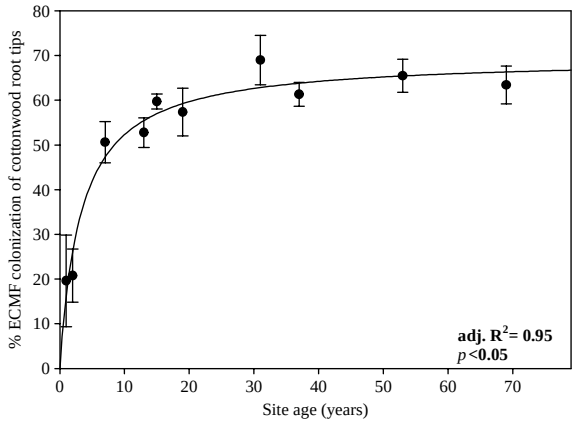

Fig. 4. Changes in percent ectomycorrhizal colonization of cottonwood root tips in October 2004 across the Nyack chronosequence fitted along a single rectangular two parameter hyperbolic model ($y = ax/(b+x)$), where $a = 64.49$ and $b = 3.29$ (mean ± standard error).

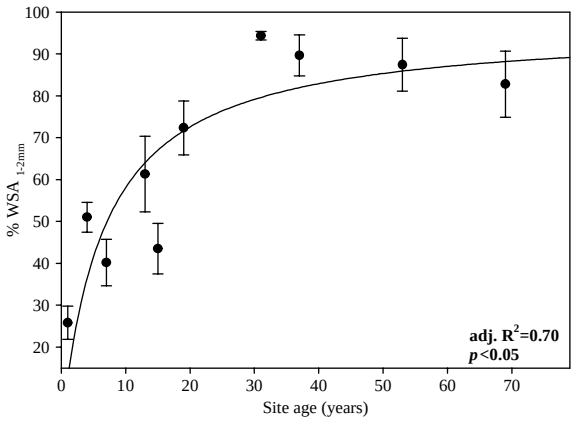

Fig. 6. Change in percent water stability of the l–2 mm aggregate size class across the Nyack chronosequence fitted along a single rectangular two parameter hyperbolic model ($y = ax/(b+x)$), where $a = 96.67$ and $b = 6.63$ (mean ± standard error).

two-parameter hyperbolic model (adj. $R^2 = 0.70$, $p < 0.05$, equation presented in figure legend). Again, this model describes a rapid increase to a stable level. The greatest increase in the percent of WSA_{1-2mm} occurred within the first 30 yr of site development, after which it remained relatively stable with a slight decline towards the oldest sites. There was no significant correlation between percent WSA_{1-2mm} and AMF soil hyphal length, but aggregate stability increased rapidly during the period where AMF were most abundant.

Discussion

This is the first documentation of change in abundance of two ecologically important mycorrhizal groups during development of floodplain soil along an unregulated river. Our study supports our prediction that abundance of AMF is greatest during early site development (1–13 yr) and then declines. We also found a steady increase in ECMF abundance throughout the chronosequence as predicted.

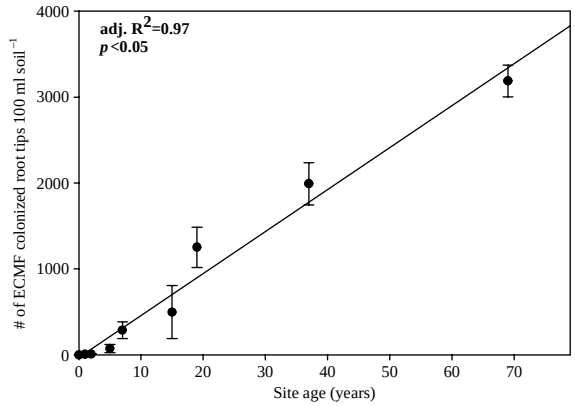

Fig. 5. Changes in abundance of ectomycorrhizae in soil as determined by the number of ECMF colonized root tips in 100 ml bulk soil at sites in August 2005 across the Nyack chronosequence fitted with a linear model ($y = y0 + ax$) where $y0 = -62.35$ and $a = 59.4$ (mean ± standard error).

This is similar to the pattern of AMF and ECMF in other temperate and boreal systems (Johnson et al. 1991, Boerner et al. 1996, Barni and Siniscalo 2000, Treseder et al. 2004), with this study the first to measure fine root colonization, mycorrhizal inoculum potential, and AMF hyphal length together across successional time. While these findings are similar to other systems with a significant ECMF hosting component, dynamics of mycorrhizae in other riparian systems lacking ECMF hosts (i.e. deserts, prairies) may be different. ECMF colonization of cottonwood roots increased much more rapidly in early succession than expected. Early proliferation of AMF and subsequent decline suggests that some ecosystem contributions of AMF may be diminished if river regulation reduces early site deposition and forests progress to host ECMF dominated soils.

Potential consequences of a decline in AMF abundance during succession

The ecosystem contributions of AMF, insofar as they are a function of inoculum potential and soil hyphal length, might be attenuated if deposition of new sediment is reduced through river regulation. AMF facilitate seedling establishment by allowing them greater access to limiting nutrients during recruitment (van der Heijden 2004). The lack of open sites created by disturbance is often cited as a factor limiting recruitment of cottonwoods (Karrenberg et al. 2002). In addition, variation in AMF inoculum potential through time may affect recruitment of other plant species that depend on AMF, possibly favoring plants with obligate AMF associations around 10 yr after disturbance, when AMF inoculum potential peaks (Fig. 2). Additionally, the presence of AMF can strongly affect plant community composition and productivity (van der Heijden et al. 1998, Rillig 2004), which could ultimately affect floodplain biodiversity and primary productivity. Although not documented, transport of mycorrhizal inoculum downstream during floods that erode upstream soil systems may be an important mechanism for dispersal of fungi. Reduction in flooding could diminish the delivery of upstream

sources of inoculum, thus also affecting plant communities downstream. Finally, AMF hyphae are significant contributing factors to soil stabilization and subsequent carbon storage (reviewed by Rillig and Mummey 2006). Despite a lack of correlation between AMF and % WSA_{1-2mm} across the whole chronosequence, our data show a rapid increase in this aggregate size class during early site development, which could be a product of AMF abundance in young soils; however, changes in organic matter content and clay accumulation during succession would also contribute to aggregate formation. Yet, soil stabilization (and hence potentially river bank stabilization) and carbon storage could be slowed with reduced AMF abundance in riparian systems.

Possible mechanisms contributing to the change between AMF and ECMF

The AMF colonization of *Populus trichocarpa* along Nyack floodplain is much lower than other observations from *Populus* and AMF in riparian areas (Jacobson 2004, Beauchamp et al. 2007). These studies assessed colonization of *P. deltoides* and *P. fremontii*, which may have a greater affinity for AMF compared to *P. trichocarpa.* These differences also may be a result of the dominant upland vegetation near the riparian areas and successional dynamics of plant communities on the floodplains. On Nyack, coniferous forests, which are almost entirely ECMF, occur in older sites and surrounding uplands. Along the southwestern rivers studied by Jacobson (2004) and Beauchamp et al. (2007), xeric vegetation surrounds the floodplain and likely associates more commonly with AMF. This suggests that the shift between AMF and ECMF in riparian areas may depend on the *Populus* species present and surrounding upland vegetation.

AMF are not lost from the system in late succession as evidenced by the moderate to high colonization of fine roots and increase in biomass of AMF hosting herbaceous plants. Nevertheless, the abundance of these fungi in soil decreases in mid to late site development. This suggests that factors other than host availability may regulate soil AMF abundance and infectivity. There are several possible mechanisms. Other studies of AMF and ECMF in riparian areas suggest that soil moisture and frequency of inundation affect relative abundances of mycorrhizal groups (Lodge 1989, Lodge and Wentworth 1990, Jacobson 2004). Furthermore, Lodge (1989) gives evidence that soil moisture can contribute to the displacement of AMF by ECMF, with AMF more abundant in drier or flooded soil, but not moist soil. Soil moisture did change across our sites but was not correlated with our measures of AMF abundance (data not presented). In Jacobson (2004), drier sites were likely colonized by xeric, AMF hosting vegetation (e.g. grasses, desert species); however, on the Nyack floodplain, drier sites tend to be older, higher elevation sites colonized by ECMF hosting conifers (Whited el al. 2007). This again suggests shifts between AMF and ECMF resulting from soil moisture changes may be very different depending on the successional dynamics of the system and the vegetation of the drier sites.

Additionally, although no other variable measured was significantly correlated with AMF hyphal length, an interesting trend was apparent. The lowest mean hyphal length, fine root AMF colonization, and near lowest inoculum potential occurred at the 31 yr old site. This site also has the greatest percent soil organic matter and surface litter. While other studies have shown additions of organic matter to stimulate AMF (Cavender et al. 2003, Nan et al. 2006), the trend we observed suggests that litter quality may be at least as important as quantity to AMF. The increased organic matter and litter could have stimulated organisms that compete with AMF. Another explanation may be that the chemistry of cottonwood litter may suppress AMF. *Populus* foliage contains soluble phenolic compounds, some of which can inhibit fungal spore germination and hyphal growth (Wacker et al. 1990, Schimel et al. 1998, Isidorov and Vinogorova 2003). Other fungi including ECMF have more complex extracellular enzyme systems capable of degrading these compounds and may be less affected (Münzenberger et al. 2003). Piotrowski et al. (2007) documents the inhibition of AMF colonization by the AMF community of the Nyack floodplain by litter and litter leachates from *P. trichocarpa.* Nevertheless, other factors also change concomitantly with time (Table 2 and 3), making it difficult to isolate any one main cause.

Ectomycorrhizal fungi do not decline at any point across this chronosequence. While the abundance of ECMF (as indirectly measured through the soil density of colonized cottonwood root tips) steadily increased throughout the chronosequence, percentage colonization of cottonwood roots by ECMF increased rapidly to near maximum within the first five years. This suggests that ECMF disperse quickly to new sites and that their abundance is strongly influenced by the presence of ectomycorrhizae hosting root tips. Increasing soil organic matter and litter accumulation may contribute to ECMF proliferation, which supports Read' s (1991) hypothesis when applied to successional systems. This hypothesis concerns the distribution of mycorrhizal types across ecosystems and postulates that as soil nutrients occur in more organic forms, the preferred mycorrhizal association will be one that can better 17 access these organic forms; hence, AMF are the preferred association in low altitude, low latitude, and early successional soils, whereas ECMF, capable of accessing organic forms of nitrogen, are the preferred association at higher altitudes, latitudes, and older sites with greater soil organic matter (Read 1991).

These data increase our sparse knowledge of the belowground component of a threatened type of ecosystem and offer an important factor to consider in managing and restoring riparian ecosystems. Our examination of the Nyack riparian chronosequence represents the first documentation of a change in mycorrhizal groups within a floodplain system and reveals a pattern that largely adheres to other observations of changes between AMF and ECMF abundance during plant community succession in temperate and boreal systems, but on a faster time scale. River management is an enterprise of increasing global significance (Bernhardt et al. 2005). River regulation may not always affect AMF community composition (Beauchamp et al. 2007), but the overall abundance of these fungi may be strongly affected. In this riparian system, regular

flooding events appear to be critical for maintaining AMF, without which soils may progress to dominance by ECMF within a relatively short period of time.

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