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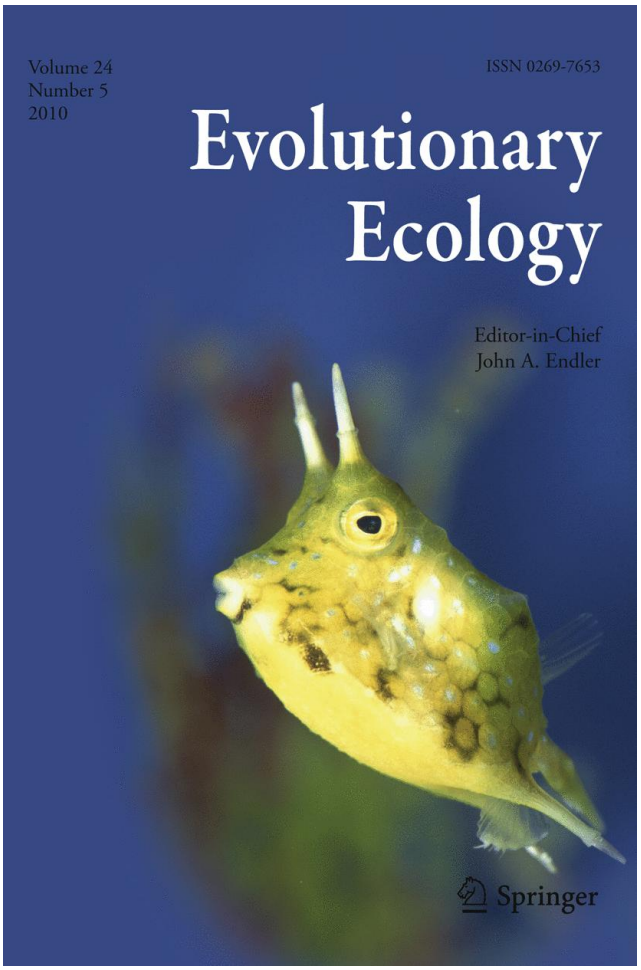

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ORIGINAL PAPER

Soils as agents of selection: feedbacks between plants and soils alter seedling survival and performance

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Abstract Soils are one of the first selective environments a seed experiences and yet little is known about the evolutionary consequences of plant-soil feedbacks. We have previously found that plant phytochemical traits in a model system, *Populus* spp., influence rates of leaf litter decay, soil microbial communities and rates of soil net nitrogen mineralization. Utilizing this natural variation in plant-soil linkages we examined two related hypotheses: (1) *Populus angustifolia* seedlings are locally adapted to their native soils; and (2) Soils act as agents of selection, differentially affecting seedling survival and the heritability of plant traits. We conducted a greenhouse experiment by planting seedlings from 20 randomly collected *P. angustifolia* genetic families in soils conditioned by various *Populus* species and measured subsequent survival and performance. Even though *P. angustifolia* soils are less fertile overall, *P. angustifolia* seedlings grown in these soils were twice as likely to survive, grew 24% taller, had 27% more leaves, and 29% greater above-ground biomass than *P. angustifolia* seedlings grown in non-native *P. fremontii* or hybrid soils. Increased survival resulted in higher trait variation among seedlings in native soils compared to seedlings grown in non-native soils. Soil microbial biomass varied significantly across soil environments which could explain more of the variation in seedling performance than soil

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texture, pH, or nutrient availability, suggesting strong microbial interactions and feedbacks between plants, soils, and associated microorganisms. Overall, these data suggest that a "home-field advantage" or a positive plant soil feedback helps maintain genetic variance in *P. angustifolia* seedlings.

Keywords Genetic variation · Local adaptation · Narrowsense heritability · Plant-soil feedback · *Populus* · Selection · Soil microbial communities

Introduction

Plant-soil feedbacks are observed when plants cause specific changes to soils and plants then have specific responses to those changes in soils. Feedbacks can vary from positive to negative and may have profound consequences for specific plant traits, plant community composition, and overall fitness (Van der Putten et al. 1993; Bever 1994; Bever et al. 1997; Mills and Bever 1998; Ehrenfeld et al. 2005; Kardol et al. 2006, 2007; Kulmatiski et al. 2008). There are many examples of how negative feedbacks between plants and soils structure plant communities and support species coexistence. For example, localized accumulations of pathogens may inhibit the growth of the dominant species offspring encouraging seedling establishment further away from the parent species (Janzen 1970; Connell 1971; but see Hyatt et al. 2003). Conversely, soil communities have been shown to be important in increasing plant growth through positive feedbacks where symbionts such as mycorrhizal fungi and nitrogen-fixing bacteria are commonly found (Bever 2003). Although belowground interactions are difficult to disentangle, it is clear that associated soil microbial communities and the processes they mediate affect plant community dynamics (Mills and Bever 1998; Bever 2003; Reynolds et al. 2003; Kardol et al. 2006, 2007; Reinhart and Callaway 2006).

While ecological feedbacks linking above- to belowground processes at the species or functional scale have been examined (Bever et al. 1997; Binkley and Giardina 1998; Northup et al. 1998; Bartelt-Ryser et al. 2005; Bezemer et al. 2006), few studies have demonstrated how genetic variation affects plant-soil linkages and the fitness consequences of these linkages. Of those studies that have examined the plant genetic basis to plant-soil linkages (Schweitzer et al. 2004; Madritch et al. 2006; Fischer et al. 2007), results generally indicate that variation in plant species traits condition the soil beneath those plants through inputs of leaf litter, roots or root exudates. Whether the plant genetic basis to plant-soil linkages conditions the soil to such an extent that it affects the fitness and performance of seedlings of those plants has not been tested. If plant-soil feedbacks have fitness consequences on the plants in subsequent generations there are at least two possible outcomes: (1) local adaptation to the parental soil type may develop; and (2) differential fitness consequences may result in changes to quantitative genetic variance.

In *Populus*, genetic-based plant traits, such as leaf chemistry or productivity, have been found to influence both community and ecosystem processes in diverse systems (reviewed in Whitham et al. 2006). For example, in a *Populus* hybridizing system it has been shown that *Populus* species (*P. fremontii* and *P. angustifolia*) and their natural hybrids, at both the stand and individual scale, can vary up to 10-fold in overall productivity (above- and belowground; Fischer et al. 2006, 2007), and leaf and litter chemistry (Schweitzer et al. 2004, 2008a; Rehill et al. 2006). This variation in plant phenotype contributes to the legacy effects of plants on soils by altering soil microbial

communities (Schweitzer et al. 2008a), rates of root turnover (Fischer et al. 2006, 2007), leaf litter decomposition, and net rates of nitrogen (N) mineralization and nitrification (Schweitzer et al. 2004). What has not yet been demonstrated is whether the effects of those plant traits on their associated soils are strong enough to impact the growth or survival of subsequent generations of seedlings and act as agents of selection (sensu Brady et al. 2005; Ellis and Weis 2006; Murren et al. 2006; Sambatti and Rice 2006). For example, variation in *Populus* spp. in foliar condensed tannins (CT) and nutrient content in the leaves alters rates of leaf litter decomposition beneath these species, as well as in adjacent streams (Schweitzer et al. 2004; LeRoy and Marks 2006). Moreover, foliar condensed tannins explained 55–65% of the variation in rates of net N mineralization in the soil resulting in different rates in the amount of available nutrients for seedlings and adjacent plants to use. Specifically, *P. angustifolia* genotypes have 30–90% higher levels of foliar CT, resulting in slower rates of net N mineralization and reduced N availability in soils than *P. Fremontii* genotypes, making soils beneath *P. angustifolia* less fertile. Differences in soil fertility can result in shifts in resource allocation among trees. For example, reduced soil fertility has been shown to stimulate increased fine root production in more nutrient limited soils (Fischer et al. 2006) and increased aboveground growth in more nutrient rich soils. Thus you would expect *P. angustifolia* seedlings to be more productive in more fertile soils (low CT inputs) and less productive in the less fertile soils (high CT inputs).

The differences in leaf chemistry, seed traits, leaf litter decomposition, and microbial communities that are apparent within and among stands of *Populus* spp., make them a model tree species to examine hypotheses regarding genetic-based plant-soil feedbacks (Schweitzer et al. 2004, 2008a; Rehill et al. 2006; Whitham et al. 2006). Here, we examined the effects of variation in pools of carbon (C), N microbial biomass N, and soil physical structure, pH and microbial community composition (based on PLFA biomarkers) of soils within forest stands that differ in their *Populus* species composition to determine if the effects of the plants on soils can impact the fitness and performance of genetic families of *Populus angustifolia* seedlings. Using field soils collected from 12 natural forest stands varying in their composition of *Populus* species and seed from 20 *P. angustifolia* genetic families, we examined the hypothesis that legacy effects from plant-soil linkages (i.e., differences in litter quality, quantity, decomposition and associated microbial communities) can impact the survival and performance of *P. angustifolia* seedlings, such that variation in forest composition will differentially condition the soils and therefore feed back to affect *P. angustifolia* seedling fitness. Planting seedlings from 20 *P. angustifolia* families into soils conditioned by other species and crosstypes (i.e., one 'home' and two 'away' soils) allows us to test the importance of local adaptation (at broad scales) on the fitness and performance of seedlings as well as determine the role of soils as selective agents. We predict soil origin differentially affects seedling fitness and performance where high mortality leads to low genetic variation and low mortality leads to high genetic variation in plant performance traits. Recent studies suggest that positive plant-foil feedbacks occur more commonly when plant species are clonal and are distributed in temperate ecosystems (Hyatt et al. 2003). Because *P. angustifolia* is a highly clonal species in temperate ecosystems we expected that when seedling families were grown in non-native soils, *P. angustifolia* seedlings would have higher mortality and thus lower genetic variation. Second, we hypothesized that the variation in soils (both biotic and abiotic) due to the legacy effects of plant inputs and differential plant-soil feedbacks will vary enough to influence rates of mortality and performance across 20 *P. angustifolia* genetic families (as estimated with half-sibling, open-pollinated families).

Materials and methods

We utilized a well-studied *Populus* system to examine the extended effects of plant-soil feedbacks on the next generation of seedlings to determine if legacy affects of previous plant conditioning influence seedling survival, fitness and trait variation. *Populus fremontii* S. Watson and *P. angustifolia* James hybridize naturally along an elevation gradient following the Weber River, Utah, USA (41.28 N, 112.08 W) creating three distinct zones with stands of trees comprised of pure *P. fremontii* (lowest elevation, <1,100 m), pure *P. angustifolia* (high elevation, >2,500 m) and hybrid zone stands (overlapping elevations) in which all tree types are sympatric (both pure species and F_1 and backcross hybrids co-occur; Keim et al. 1989) and dominant in the stands. As previously mentioned, soils in these forests vary in soil microbial communities, root turnover, litter decomposition and annual rates of net N mineralization and nitrification depending upon the dominant overstory *Populus* species or crosstype (Schweitzer et al. 2004, 2008a, Fischer et al. 2006, 2007, in press). *Populus angustifolia* zones are typically characterized by having slow rates of litter decomposition, high rates of microbial immobilization, slow rates of net N mineralization and nitrification relative to stands dominated by *P. fremontii*, with stands of *P. fremontii* × *P. angustifolia* natural hybrids intermediate. All soils in these forests are alluvial, sandy-loams characterized as mesic Fluventic Haploxeroll, mesic Entic Haploxeroll, and frigid Cumulic Haploborolls across the *P. fremontii*, hybrid, and *P. angustifolia* zones, respectively. The soils are 64–73% sand and vary in soil pH from 7.2 to 7.3, across the *P. fremontii*, hybrid, and *P. angustifolia* zones, respectively (Schweitzer et al. 2002).

Soil collections

Soil was collected from within the drip-line of 15 randomly selected cottonwood trees from four forest stands within each of the three zones (12 collection sites total; *P. fremontii*, hybrid, and *P. angustifolia* zones). Soils were collected from the 0 to 15 cm depth and all plants and large roots (>1 mm in diameter) were removed. A sub-sample of the pooled soil from each individual site was placed in a cooler with ice before transported to the laboratory where a portion of it was immediately frozen for later microbial community analyses (see methods below). The four samples from each site (i.e. forest stand) were then combined and thoroughly mixed (Pro-Grow Soil mixer SM10-3 2EQ2027, Brookfield, WI USA) to create a homogenized soil that was representative of each of the three locations into which seedlings would be planted.

We measured several microbial aspects of the soils including microbial biomass N and microbial community composition using phospholipid fatty acid analysis (PLFA) to determine if differences in soil microorganisms occur between the three zones and are correlated with seedling performance and survival. All soil samples used for microbial and chemical analysis were kept cold (4°C) until processed (within 5 days) and sieved (<2 mm) to remove any remaining coarse fragments and roots. From these samples we measured microbial biomass N with the Chloroform Fumigation Extraction method (Haubensak et al. 2002). A sub-sample of fresh field soil (~25 g) was extracted with 50 ml 0.5 M K_2SO_4, shaken, gravity-filtered with Whatman filter paper no. 1 (leached with deionized water and K_2SO_4) and stored in a freezer until chemical analysis. Another ~25 g sub-sample was exposed to chloroform for 5 days in a glass vacuum desiccator with 30 ml ethanol-free chloroform. Following the fumigation period and removal of the chloroform with repeated evacuations, the samples were extracted with 50 ml of 0.5 M

K_2SO_4 in the same approach as above. Total microbial biomass N was determined on the thawed extracts with a micro-Kjeldahl digestion followed by colorimetric analysis using the salicylate method on a Lachat AE auto-analyzer (Lachat Industries, Inc., Loveland, CO, USA). Another fraction of each field soil was immediately frozen (−80°C) and then freeze-dried to evaluate microbial community composition with PLFA biomarkers using the method of White and Ringleberg (1998). The freeze-dried soil was extracted with a phosphate-buffered chloroform–methanol solvent (Bligh and Dwyer 1959) and separated into functional classes of signature fatty acids after methylation of the polar lipids and analysis by gas chromatography (White et al. 1979; Agilent Technologies GC-Mass Spectrometer [6890N GC/5973N MSD] Palo Alto, CA, USA). Phospholipid fatty acids are distinctive to major taxonomic groups (e.g., gram positive and gram negative bacteria, fungi; White et al. 1979; Zelles 1999; Leckie 2005) and are valuable for differentiating treatment patterns for broad microbial community assessment (Ramsey et al. 2006).

Another sub-sample of field moist soil was oven dried (48 h at 105°C) to determine soil water content. All microbial biomass and PLFA values are expressed on an oven-dry mass basis. The nutrient status of each soil was then evaluated by measuring the soil organic carbon (C) and total nitrogen (N) content of the soil. Soil sub-samples were air-dried and ground to a fine powder before the soil C and N were quantified. The samples were run on a Thermo-Finnigan Delta^{plus} Advantage gas isotope-ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer (Thermo Fisher Scientific, Inc. Waltham, MA, USA) at the Colorado Plateau Stable Isotope Laboratory (http://www.mpcer.nau.edu/isotopelab/).

Variation in soil characteristics were analyzed with ANOVA using location of soil origin as a fixed effect (with SAS-JMP 5.1). We used Non-metric Multidimensional Scaling ordination (NMDS; Minchin 1987) to examine soil microbial communities (PLFA) at each site based on the Bray-Curtis distance (Faith et al. 1987). We used Analysis of Similarity (ANOSIM) to examine the relationship of microbial community composition to soil origin using Primer-E (version 6.1.8).

Seed collection

Along the Weber River it has previously been determined that plants influence their associated soils (Schweitzer et al. 2004, 2005, 2008a, b; Fischer et al. 2006, in press), therefore we utilized this river canyon as a model system to determine how the effects of previously conditioned soils (described above) may impact the fitness and performance of *Populus angustifolia* seedlings. To this end we planted successfully germinated *P. angustifolia* seedlings from half-sibling, open-pollinated genetic families into each of the soils described above. We used open-pollinated seedlings to estimate fitness and performance traits and narrow-sense heritability, an approach that has commonly been utilized in plant breeding programs globally (Falconer and McKay1996; Potts and Jordan 1994). *Populus* are dioecious, obligately outcrossing species (Braatne et al. 1996), therefore each tree represented a single mother and the offspring (seeds) were most likely half-siblings. Using open pollinated seeds assumes a random mix of pollen from male trees and a random chance of pollen from any male tree fathering any particular seedling. To reduce the potential of dominance effects due to non-random pollination, seeds were collected from at least 10 randomly chosen open pollinated catkins (from random branches in the tree) from 20 different female *P. angustifolia* genotypes in the *P. angustifolia* native range, unfortunately *P. fremontii* seeds were not available to use in this study at this time. To avoid the possibility of collecting seedlings from clonally related mothers, female trees

used in this study were separated by at least 50 m and up to 25 km. The seeds were removed from the catkins by hand and gently sieved with a 4 mm sieve to remove the seed hairs. The seeds were chosen randomly and germinated on distilled water moistened filter paper in large Petri plates in a growth chamber where humidity (~15%), temperature (22°C), and light were standardized relative to field means (Schweitzer et al. 2004). Successfully germinated seeds (10 from each family) were randomly chosen and planted into bookplanters with each of the three soils (soil from the composites of *P. fremontii* stands, *P. angustifolia* stands, hybrid zone stands; 20 seed families × 3 soil types × 10 seeds each for 600 seedlings total).

Seedling performance

Seedling mortality, height, leaf length, and number of leaves were measured at multiple dates during the growing season, while the seedlings developed under uniform environmental greenhouse conditions (i.e., temperature and a weekly watering schedule of 3 L/bookplanter). Leaf biomass was assessed on the seedlings after senescence in the fall (~November) to determine seedling above-ground productivity in response to growing in the different soils. Leaf biomass data was calculated by individually collecting all senescent leaf litter from each seedling. Leaf litter was collected into filter paper cones wrapped around each seedling, dried for 48 h (at 70°C) and summing the total biomass. During the growing season all bookplanters were randomly repositioned in the greenhouse every 2 weeks to minimize greenhouse micro-site effects on seedling performance. Fitness and performance measurements taken from the November 2005 collection date (peak productivity) were used for all analyses.

Analyses

To test for differences in fitness and performance we used restricted estimated maximum likelihood (REML) in SAS-JMP 5.1. The statistical model included seed family and replicate as a random effect, soil origin as a fixed effect and the interaction of soil and seed family as a random effect to determine seedling performance across the soils from varying origin. The significance of seedling family and the interaction term was tested with a log-likelihood ratio test (Conner and Hartl 2004).

Our collection of 20 genetic families allowed us to calculate the narrowsense heritability of the response of seedlings to the soils of different origin (Falconer and McKay 1996; Conner and Hartl 2004). We used standard quantitative genetic methods to relate the observable components of phenotypic variance (i.e., mortality and various performance traits) to the measurable components of genetic variance. We estimated the narrowsense heritability (h^2_{op}) using the following equation:

$$h^2_{\text{op}} = 4 \times \left[\sigma^2_{\text{among families}} / \sigma^2_{\text{total}}\right],$$

where the total variance was measured as

$$\left[\sigma^2_{\text{among families}} + \sigma^2_{\text{error}}\right],$$

where σ^2 denotes the variance of a given component. The h^2_{op} is a measure of the proportion of variance in the plant phenotype that is attributable to additive genetic variance with the common assumptions associated with open pollinated progeny (Falconer and

McKay 1996). Variance components and standard error were taken directly from the REML statistical outputs. Heritability was calculated for each performance variable and was considered to be significant when the log-likelihood tests indicated significant differences among families within each soil type (Conner and Hartl 2004).

Results

Soils characteristics

We found significant differences in chemical and biological properties between the soils based on the composition of plants that had previously conditioned them (Table 1), however we found no significant differences in soil texture, suggesting that physical differences in the soils probably did not influence the seedling performance and survival (Table 1). Contrary to previous studies (Schweitzer et al. 2004) we found no significant differences in soil organic C and total N between the three locations of soil origin, although they did differ by 38 and 58%, respectively. However, we did find a significant difference (24%) in the C:N ratio as well as a significant, two-fold difference in microbial biomass N pools among the three zones of soil origin. *P. angustifolia* soils had the highest C:N ratio and the highest microbial biomass N, with hybrid soils intermediate and *P. fremontii* soils the lowest, indicating that the soil previously conditioned by *P. angustifolia* trees were the most N limited (Schweitzer et al. 2004, 2008b).

We found no difference in microbial community composition by soil origin (ANOSIM: $R = 0.073$, $P = 0.318$). Only one individual PLFA varied by soil origin, C16:1ω7t

Table 1 ANOVA results indicating soil characteristics for stands of gallery forest (sites) in the *P. fremontii*, hybrid and *P. angustifolia* zones

Zone	Site[a]	% Sand/silt/clay	% C	% N	C:N	Microbial biomass N (mg N/kg)
P. fremontii[b]		64/23/11[a]	2.86[a]	0.14[a]	20.62[a]	52.12[a]
	RR	61/25/14	2.74	0.12	22.95	63.72
	Levy	76/18/7	1.91	0.09	20.62	50.31
	H3	69/20/11	3.46	0.17	20.32	79.26
	Site 2	56/31/13	3.32	0.18	18.61	42.32
Hybrid[b]		69/21/10[a]	3.81[a]	0.21[a]	18.14[a]	67.96[a]
	Site 9	78/14/7	5.19	0.27	18.89	67.57
	BSH	55/31/13	3.58	0.20	17.75	98.19
	NU1	59/30/11	2.50	0.13	18.41	43.94
	FSH	82/10/8	3.98	0.23	17.42	62.13
P. angustifolia[b]		73/20/7[a]	6.48[a]	0.24[a]	26.8[b]	105.59[b]
	CO1	84/10/6	3.02	0.10	28.80	73.39
	CO2	69/24/7	3.77	0.14	26.42	86.85
	Tag	70/22/8	12.27	0.40	30.95	140.30
	Hen	69/23/8	6.87	0.32	21.60	156.51

[a] Mean values for a site ($n = 15$/site)

[b] Lowercase online alphabets, for each response variable, indicate significance at the $\alpha = 0.05$ level among zones as measured with at Tukey's HSD

[general bacteria (Wilkinson 1988); $F = 7.94$, $P = 0.015$]. The abundance of this biomarker was 39% lower in the *P. angustifolia* soils than in *P. fremontii* soils; the hybrid zone was intermediate in the abundance of this biomarker. These results suggest that, while the soils are similar in texture, pH and total C and N, the soils from different zones vary in the ratio of C:N and microbial N pools, with *P. angustifolia* soils differing the most.

Survival

Consistent with a positive plant-soil feedback hypothesis, *P. angustifolia* seedlings showed the highest survival in *P. angustifolia* soils, despite these being the soils which are the most N limited. Overall, there was a significant seed family, and seed family by soil origin interaction for seedling survival, indicating a genetic basis for survival, however specific seed families which survived varied among soil types (Table 2). After starting with equal numbers of living seedlings from all 20 seed families, the lowest mortality of *P. angustifolia* seedlings was in the native *P. angustifolia* soils and the highest mortality of seedlings was in the *P. fremontii* soils, which is furthest from the seed origin (Fig. 1; Table 2). *P. angustifolia* seedlings grown in *P. fremontii* zone soils had 32% mortality while those grown in hybrid zone or *P. angustifolia* soils had 15 and 17% mortality, respectively. Importantly, post-hoc analysis showed a significant genetic effect for seedling mortality when seeds were planted in *P. fremontii* and hybrid soils, but not when seedlings were planted in *P. angustifolia* soils (Table 3). These results indicate that seedling survival was greatest in *P. angustifolia* soils and all families survived equally well. In the hybrid soils, seedling survival was equivalent to *P. angustifolia* soils but all families did not survive equally well, and in *P. fremontii* soils seedling survival was lowest with differential survival among families. These results are consistent with the hypothesis that *P. angustifolia* seeds are locally adapted to *P. angustifolia* soils.

Performance

Consistent with the hypothesis of positive plant-soil feedbacks and local adaptation, we found that *P. angustifolia* seedling performance was also best in *P. angustifolia* soils (Table 3). Among the different response variables measured, when averaged across all families in each soil origin, performance was significantly greater among *P. angustifolia*

Table 2 Restricted estimated maximum likelihood results (REML) for mortality and performance values of *P. angustifolia* families growing in soils collected from three locations of origin (natural *Populus fremontii*, hybrid [*P. fremontii* × *P. angustifolia*], and *P. angustifolia* stands)

	Mortality		Height		Leaf number		Leaf length		Biomass	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Soil origin (F)	6.33	0.0043	24.20	0.0001	8.14	0.0013	10.15	0.0003	4.27	0.021
Family (χ^2)[a]	0.46	0.25	2.88	0.044	0.28	0.30	0.88	0.17	1.98	0.08
G × E[b] (χ^2)[a]	10.19	0.0007	3.17	0.038	3.86	0.025	9.74	0.0009	6.15	0.0066

[a] χ^2—numbers in the *F* column of the family row represent chi-square values

[b] Family × Soil origin interaction. The degrees of freedom for all tests are: whole model = 59, Soil origin = 2, Family = 19, G × E = 38

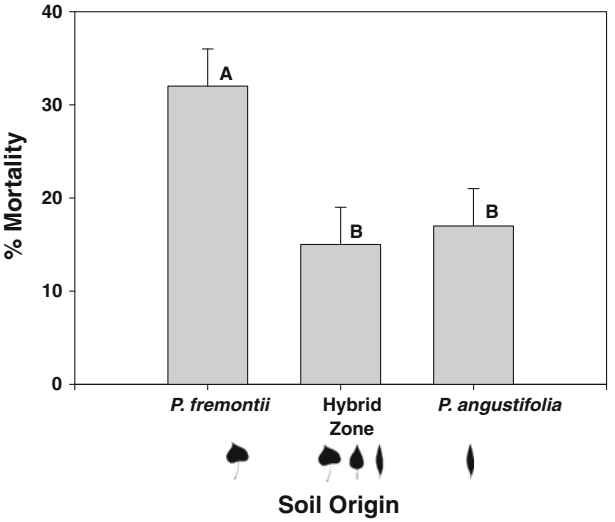

Fig. 1 On average, the percentage mortality of seedlings from 20 *Populus angustifolia* genetic families is highest when grown in *P. fremontii* soil and lower when grown in *P. angustifolia* and hybrid (*P. fremontii* × *P. angustifolia*) soil (± one standard error) different letters resemble significant differences across soil origins (determined by Tukey's HSD post-hoc test)

Table 3 Likelihood ratio tests and narrowsense heritability (h^2_{op}) for 20 *P. angustifolia* seed families grown in soils collected from field sites dominated by *P. angustifolia* trees, *P. fremontii* trees or hybrid zone soils in which both pure species and their hybrids grow together

Soil origin	Trait	X^2	P	h^{2a}
P. angustifolia overstory	Height	14.12	0.0001	0.20 ± 0.1[b]
	Leaf biomass	12.6	0.0002	0.18 ± 0.09[b]
	Leaf length	19.4	0.0001	0.23 ± 0.11[b]
	# of leaves	11.6	0.0001	0.17 ± 0.09[b]
	Mortality	0	0.5	0
Hybrid overstory	Height	2.37	0.06	0.07 ± 0.06
	Leaf biomass	6.55	0.005	0.13 ± 0.08[b]
	Leaf length	4.13	0.02	0.10 ± 0.07[b]
	# of leaves	0.53	0.23	0.03 ± 0.05
	Mortality	7.55	0.003	0.12 ± 0.06[b]
P. fremontii overstory	Height	0.29	0.3	0.02 ± 0.05
	Leaf biomass	0.37	0.27	0.04 ± 0.06
	Leaf length	0.17	0.34	0.02 ± 0.05
	# of leaves	1.52	0.11	0.07 ± 0.08
	Mortality	14.1	0.0001	0.17 ± 0.08[b]

[a] ± One standard error

[b] Represents heritability estimates that are significantly different from zero

seedlings grown in their native soil (Fig. 2). Seedling leaf biomass was 29% greater on *P. angustifolia* seedlings grown in their native soil than *P. fremontii* soil, with hybrid soils intermediate. *P. angustifolia* seedlings grown in *P. angustifolia* soil were 24% taller

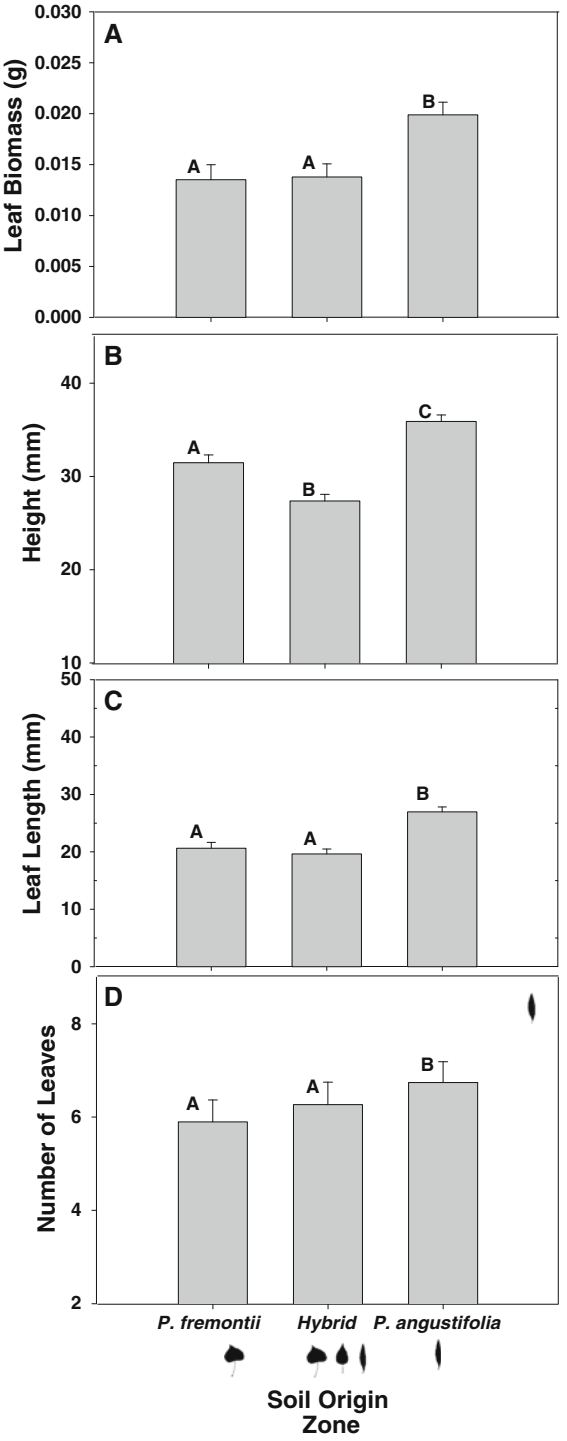

Fig. 2 On average *P. angustifolia* seedlings performed better when grown in *P. angustifolia* soil. Performance measurements above-ground biomass (**a**), height (**b**), leaf length (**c**), and number of leaves (**d**) of *P. angustifolia* seedlings grown in soils conditioned by *P. fremontii* trees, hybrid (*P. angustifolia* × *P. fremontii*) trees, and *P. angustifolia* trees. Different letters indicate significant differences when averaged across all 20 families (± one standard error)

than seedlings grown in hybrid soils and 13% taller than seedlings grown in *P. fremontii* soil (Fig. 2). Leaves were 27% longer on *P. angustifolia* seedlings grown in their native soil than *P. fremontii* soil, with hybrid soils intermediate. There were 11% more leaves on *P. angustifolia* seedlings grown in their native soil than *P. fremontii* soil, with hybrid soils again being intermediate. While fitness and performance were higher, on average, when *P. angustifolia* seedlings were grown in their native soil, the significant interaction term indicated that seed family performance varied by soil origin. In combination with the results that *P. angustifolia* seedlings survived better in *P. angustifolia* soils relative to *P. fremontii* soils, these results suggest that genetic variance (i.e., narrowsense heritability) for *P. angustifolia* seedling performance traits may also vary by soil origin.

Heritability

Because seedling survival was highest in *P. angustifolia* soils and all families survived equally well, the highest variation (h^2) in seedling performance traits should occur in the *P. angustifolia* soils. Furthermore, heritability of these traits should decline in the hybrid soils where survival was still high (but not all families survived equally well) and be lowest in the *P. fremontii* soils where survival was significantly lower with far fewer families doing well. Consistent with this hypothesis, we found that all *P. angustifolia* seedling traits were heritable in the *P. angustifolia* soils and that heritability ranged from 0.17 to 0.23, depending upon the trait (Fig. 3; Table 3). As expected genetic variation for the measured performance traits declined in the hybrid soils where only three traits showed additive genetic variation. There was no additive genetic variation for any of the performance traits we measured when *P. angustifolia* seedlings were planted in *P. fremontii* soils as mortality was the greatest in these soils.

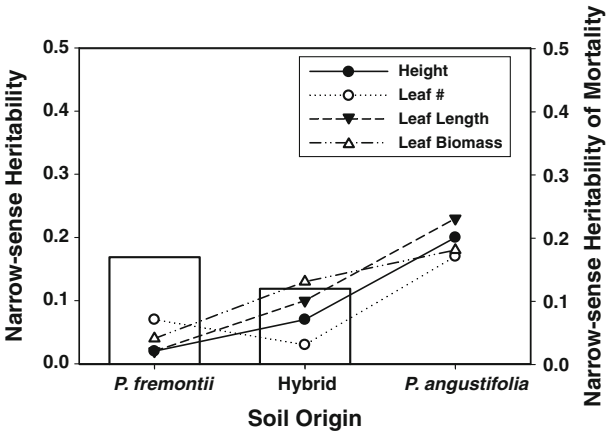

Fig. 3 Seedling mortality negatively affects narrow-sense heritability. In *Populus angustifolia* soils seedling survival was high and all seedling families survived equally well and genetic variation for all performance traits are the highest. In *P. fremontii* soils where seedling survival was lowest and there was significant variation among seedling families for survival, genetic variation for performance traits was low. Soil origin is on the x axis. Bars represent the narrow-sense heritability of seedling mortality from Table 3 (i.e. here was no genetic variation for mortality of *P. angustifolia* seedlings in *P. angustifolia* soil). Symbols represent the narrow-sense heritability of seedling performance from Table 3

Discussion

While studies have examined plant-soil feedbacks, few have been placed in an evolutionary framework of natural selection and heritability, perspectives that are critical if we are to understand evolutionary processes in the context of ecosystem science. Dominant plant species (Ellison et al. 2005) can have legacy effects on soils that can feed back to affect the plants own fitness (Bever et al. 1997; Mills and Bever 1998; Binkley and Giardina 1998). Here, we found that this effect holds even with closely related species and their hybrids growing sympatrically. Our data show a "home field advantage" or positive feedback for *P. angustifolia* seedlings when grown in the soil that had been previously conditioned by *P. angustifolia* trees, as opposed to soils in which other closely related species and their hybrids were grown. We found that seedlings grown in their native soil have higher survival and higher performance relative to seedlings grown in soils conditioned by other *Populus* species even though these soils were the most N limited (i.e., highest C:N ratio and highest microbial N pools; Schweitzer et al. 2004, 2008b; Fischer et al. in press). *P. angustifolia* seedlings grown in *P. angustifolia* soils were twice as likely to survive, grew 24% taller, had 27% more leaves, and a 29% greater total above-ground biomass relative to two other soil environments. Consistent with increased survival and performance, seedlings grown in their native soil resulted in higher trait variation (i.e., significant additive genetic variance) compared to seedlings grown in non-native soils. Because soil conditions to which the seedlings are adapted are a consequence of inputs by leaves and roots by the parent species (Schweitzer et al. 2004, 2008a, b, Whitham et al. 2006), these results suggest that local adaptation can be a consequence of plant soil feedbacks.

A rich literature on feedbacks from plant traits has demonstrated that plants can create unique environments and influence soil physical, chemical, and biotic properties that can feedback to their performance (Zinke 1962; Boettcher and Kalisz 1990; Rhoades 1997; Binkley and Giardina 1998; Smith et al. 1999; Packer and Clay 2000; Klironomos 2002; Reynolds et al. 2003; Kasurinen et al. 2005; Kardol et al. 2007, Kulmatiski et al. 2008). However, most of this research has been focused at the species or stand level. Recent data demonstrates that in long-term common environments, *Populus* genotypes can condition their associated soils to structure aspects of the microbial community and impact ecosystem processes (Madritch et al. 2006, Schweitzer et al. 2008a). We predict that the consequences of a positive feedback over time would allow for an increase in the abundance of *Populus* genetic families in areas where parent trees have conditioned the soil and a decrease in the abundance of *Populus* that originated further away (Klironomos 2002; Reynolds et al. 2003). These feedback responses will manipulate the genetic variation in *Populus* stands and their associated communities, greatly influencing the evolutionary trajectories of these ecosystems.

While at broad scales soils clearly have been shown to impact plant distributions and even dictate plant genetic structure, community composition and invasiveness (Muller 1982; Bever et al. 1997; Mills and Bever 1998; Reynolds et al. 2003; Baack et al. 2006; Kardol et al. 2006; Levine et al. 2006; Alvarez et al. 2009), quantification of the genetic basis of these feedbacks and the fitness correlations between soils and plants are rare (but see Murren et al. 2006; Sambatti and Rice 2006). Some of the best examples of soils as agents of selection are found in serpentine soil studies in which serpentine soils and their unique chemistry can select for particular plant communities and even genotypes, with evolutionary consequences (Brady et al. 2005; Ellis and Weis 2006; Murren et al. 2006; Sambatti and Rice 2006). For example, using a reciprocal transplant study, Murren et al.

(2006) found that soil chemistry and water conditions exhibit strong selective pressure on *Mimulus grattus,* a species that occurs in serpentine and non-serpentine environments. They found significant genetic variation at the family level in leaf, stem, stolon, and floral traits when grown in soils mimicking four field conditions (low or high calcium to magnesium ratios and low or high water availability). They found significant variation in survivorship and performance across the different soil conditions, reflecting the importance of phenotypic plasticity and genetic variation to the survival of this species. The results from our study support a positive plant-soil feedback hypothesis and indicate that *P. angustifolia* seedlings appear to be locally adapted to *P. angustifolia* soils, even though these soils demonstrate the lowest fertility of the three tested soil types as indicated by this study (i.e., highest C:N ratios and highest microbial immobilization) as well as previous work in this system (Schweitzer et al. 2004; Fischer et al. in press). Although in a strict sense the study of local adaptation of both Populus species requires a reciprocal transplant to test whether *P. angustifolia* and *P. fremontii* each outperform the other on their respective local soils (Kawecki and Ebert 2004), the results of the study are in line with local adaptation of *P. angustifolia*. Moreover, this study demonstrates that soils previously conditioned by *Populus* spp. can act as agents of selection influencing the genetic variance of plant traits in subsequent generations. Overall these data suggest tight feedbacks between plants and their soils which can influence plant evolutionary trajectories. Future studies are required to better understand the reciprocal feedbacks within this system as well as the generality of this effect across multiple plant species, the specific mechanisms of this effect as well as models to predict the long-term consequences at the landscape scale.

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