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The invasive species *Alliaria petiolata* (garlic mustard) increases soil nutrient availability in northern hardwood-conifer forests

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Abstract The invasion of non-native plants can alter the diversity and activity of soil microorganisms and nutrient cycling within forests. We used field studies to analyze the impact of a successful invasive groundcover, *Alliaria petiolata*, on fungal diversity, soil nutrient availability, and pH in five northeastern US forests. We also used laboratory and greenhouse experiments to test three mechanisms by which *A. petiolata* may alter soil processes: (1) the release of volatile, cyanogenic glucosides from plant tissue; (2) the exudation of plant secondary compounds from roots; and (3) the decomposition of litter. Fungal community composition was significantly different between invaded and uninvaded soils at one site. Compared to uninvaded plots, plots invaded by *A. petiolata* were consistently and significantly higher in N, P, Ca and Mg availability, and soil pH. In the laboratory, the release of volatile compounds from the leaves of *A. petiolata* did not significantly alter soil N availability. Similarly, in the greenhouse, the colonization of native soils by *A. petiolata* roots did not alter soil nutrient cycling, implying that the exudation of secondary compounds has little effect on soil processes. In a leaf litter decomposition experiment, however, green rosette leaves of *A. petiolata* significantly increased the rate of decomposition of native tree species. The accelerated decomposition of leaf litter from native trees in the presence of *A. petiolata* rosette leaves shows that the death of these high-nutrient-content leaves stimulates decomposition to a greater extent than any negative effect that secondary compounds may have on the activity of the microbes decomposing the native litter. The results presented here, integrated with recent related studies, suggest that this invasive plant may change soil nutrient availability in such a way as to create a positive feedback between site occupancy and continued proliferation.

Keywords Microbial diversity · Nutrient cycling · Biofumigation · Root exudation · Litter decomposition

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Introduction

There is increasing evidence that invasive plants can alter the diversity and activity of soil microorganisms and nutrient cycling (Klironomos 2002; Ehrenfeld 2003). Soil nutrient availability is one of the most important factors determining ecosystem stability and productivity (Chapin et al. 1996; Tilman et al. 1997). Therefore, invasive species that change soil properties can have far-reaching and potentially long-term impacts on the structure and function of ecosystems.

The degree to which an invasive plant alters soil processes is difficult to predict (Ehrenfeld 2003). Invasive plants have been shown to alter the composition of microbial communities (Angeloni et al. 2006; Stinson et al. 2006), stimulate or inhibit microbial activity (Hawkes et al. 2005; Boon and Johnstone 1997), and increase or decrease the rate of nutrient cycling in the soil (Vitousek et al. 1987;

Mitchell et al. 1997; Kourtev et al. 1999; Ehrenfeld et al. 2001). The mechanisms underlying these belowground impacts vary widely, including novel nutrient uptake strategies (Baruch and Goldstein 1999; Nagel and Griffin 2001), alteration of the physical properties of the soil environment (Duda et al. 2003; Enloe et al. 2004), input of litter with unique chemical properties (Evans et al. 2001; Ashton et al. 2005), modification of fire regimes (D'Antonio and Vitousek 1992), and the release of secondary compounds (Boon and Johnstone 1997; Callaway and Aschehoug 2000).

Alliaria petiolata (garlic mustard; Brassicaceae) was introduced to the United States in 1868 from Eurasia, and it is now widely distributed throughout the US and Canada (Cavers et al. 1979). As an obligate biennial, *A. petiolata* produces first-year rosettes that over-winter under the snow, bolt early the following spring, set seed, and die by mid summer (Cavers et al. 1979). *A. petiolata* is unusual as an invasive, herbaceous plant in that it is shade tolerant, non-mycorrhizal, and can invade the forest understory in the absence of disturbance (Meekins and McCarthy 2001). Once *A. petiolata* invades, it becomes a permanent member of the community and proliferates rapidly (Nuzzo 1999). *A. petiolata* is considered one of the most problematic invaders of North American forests, and has been found to decrease the abundance of mycorrhizal fungi in the soil and on plant roots (Stinson et al. 2006), inhibit the growth of native plants (Meekins and McCarthy 1999; Prati and Bossdorf 2004), and cause declines in the diversity of native plant communities (McCarthy 1997; Stinson et al. 2007; Rodgers and Finzi, in review). To date, however, there has been no work focusing on the impact of *A. petiolata* invasion on microbial activity and soil nutrient cycling.

Like most mustard plants, *A. petiolata* produces abundant secondary compounds (Haribal and Renwick 1998; Haribal et al. 2001; Cipollini 2002). The main group of secondary compounds present in mustard plants is glucosinolates, sulfur- and N-containing compounds that degrade into volatile cyanide molecules (Fahey et al. 2001). These cyanide molecules are known to deter herbivory (Cipollini 2002) and suppress the growth of plant (Bialy et al. 1990) and fungal species (Mayton et al. 1996). Recently, Cipollini and Gruner (2007) found that the concentration of cyanide in the tissues of *A. petiolata* is as high as 100 p.p.m. fresh weight, a level considered toxic to most vertebrates, and 150 times that of native mustard plants. Additional secondary compounds have also been identified in *A. petiolata* leaves, including defense proteins and a variety of flavonoids (Haribal and Renwick 1998; Haribal et al. 2001; Cipollini 2002).

The release of secondary compounds to the soil by *A. petiolata* has the potential to inhibit microbial activity (Mayton et al. 1996) and therefore slow nutrient cycling. How secondary compounds are incorporated into the soil is currently unknown, although previous research allows us to predict candidate mechanisms. First, in agricultural systems, mustard plants are frequently used to inhibit the activity of unwanted soil organisms through "biofumigation" (Brown and Morra 1997, Fig. 1). This widely used practice involves tilling fresh plant material into the top layer of the soil, allowing the release of volatile compounds that fumigate the soil. A similar process may occur in non-agricultural settings, if above- or belowground damage occurs to *A. petiolata* (e.g., via herbivory, Nahrstedt 1985), thereby stimulating the release of cyanogenic glycosides in and around the soil of invaded areas. Second, root exudation by *A. petiolata* may release secondary compounds directly into the soil (Fig. 1). This process has been implicated in the successful invasion of *Centaurea* spp. in the western US (Callaway and Aschehoug 2000), and root exudation by *A. petiolata* has been found to inhibit the germination of native seeds (Prati and Bossdorf 2004). Third, changes in the quantity and chemistry of litter may alter decomposition and microbial activity (Evans et al. 2001; Mack and D'Antonio 2003; Fig. 1).

It has been proposed that invasive plants may benefit from a positive feedback with their soil communities (Klironomos 2002), but that native plants may be negatively impacted by these same changes (Callaway and Aschehoug 2000; Stinson et al. 2006). Given that many invasive plants negatively impact the native plant communities they invade (D'Antonio et al. 1998; Wilcove et al. 1998; Stinson et al. 2007) and that *A. petiolata* contains abundant plant secondary compounds that have the potential to adversely affect

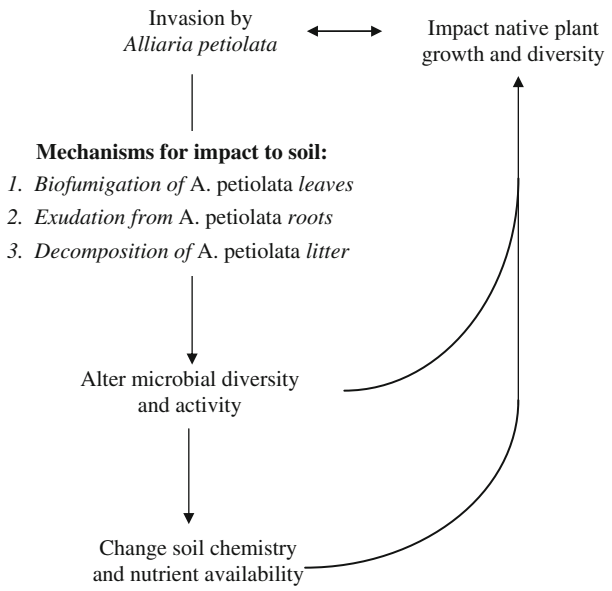

Fig. 1 Conceptual model for experiments with *Alliaria petiolata*, indicating the impacts of its invasion and the mechanisms for impacts on soil

microbial function, we predicted that invasion by *A. petiolata* would slow microbial activity and decrease nutrient availability in five forested sites differing in canopy tree species composition. To test this prediction, we compared the diversity of microbial communities and the rate of nutrient cycling in field plots that were invaded by *A. petiolata* with plots that had no *A. petiolata*. We then conducted a series of laboratory and field experiments focusing on biofumigation of the soil, root exudation, and litter chemistry as the processes controlling changes in microbial diversity and activity, and soil nutrient cycling (Fig. 1).

Materials and methods

Study sites and plot establishment

We established plots in five different sites varying in plant species composition, soil horizon development and mycorrhizal association (Table 1). The five sites included three hardwood-dominated sites, one conifer-dominated site, and a mixed site. Sites were named after their location in Connecticut and Massachusetts: Appalachian Trail (AT), Hammond Woods (HW), Pine Plantation (PP), Norfolk Land Trust (NLT), and Robin's Swamp (RS) (Table 1). The sites were approximately 40 m × 40 m each and contained substantial areas of *A. petiolata* invasion with easily identifiable adjacent areas that had not yet been invaded (V. L. Rodgers and A. C. Finzi, personal observation). The sites had been invaded by *A. petiolata* for a minimum of 2 years, and likely much longer (V. L. Rodgers and A. C. Finzi, personal observation), although the estimated age of invasion at each site was not available. In May 2005 we established twelve 1.2 m × 1.2 m plots at each of the five sites. Half of the plots were placed in areas invaded by *A. petiolata* and the other half were placed in adjacent uninvaded areas. We considered an area invaded when the density of *A. petiolata* plants exceeded 100 plants m^{-2}. The uninvaded plots were chosen to be representative of the uninvaded areas of each site and they were placed at least 2 m but not greater than 5 m from the nearest *A. petiolata* plant. The close proximity of the plots ensured that soil characteristics were similar in the invaded and uninvaded plots. Given that few other understory plants form dense monospecific patches in New England forests, the invaded plots at each site contained significantly higher numbers of plants than the uninvaded plots (Table 1). There were 60 field plots in total (5 sites × 2 invasion classes × 6 replicates). The experimental design of the field plots allowed for the analysis and comparison of undisturbed soil systems across a range of soil nutrient levels. To allow for manageable sample sizes some sites were excluded from some of the analyses as indicated below.

Microbial diversity in the field

Fungal community composition was assessed at the NLT, PP, and RS sites using length heterogeneity-polymerase chain reaction (LH-PCR) of the internal transcribed spacer region 1 (ITS1) region of rRNA. Genomic DNA was extracted from a subsample of the soil used for the community-level physiological profiles analysis using the MoBio PowerSoil DNA kit (MoBio Laboratories, Carlsbad, Calif.). Two separate DNA extractions were done for each sample. PCR products and LH-PCR profiles from these two separate extractions were kept separate until the statistical analysis stage at which point they were averaged together to represent a plot.

DNA was amplified using the fungal primers ITS1-F and ITS2 (Gardes and Bruns 1993; White et al. 1990). ITS1-F was labeled with 6-carboxyfluorescein. Each PCR reaction (25 µl) contained 25 ng of DNA template, 0.5 U of EconoTaq DNA polymerase (Lucigen, Middleton, Wis.), 1X Econotaq buffer, 400 nm of both primers, deoxynucleotide triphosphates (200 µM of each), and a PCR enhancer mix (Ralser et al. 2006). Two PCR reactions for each soil sample were conducted, pooled, and then cleaned with the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, Wis.).

An aliquot of 1 µl (approximately 30 ng) of cleaned PCR amplicons was mixed with 0.25 µl of GeneScan–500 ROX size standard and 8.75 µl formamide and analyzed on a ABI3130xl Genetic Analyzer (Applied Biosystems, Foster City, Calif.). Size and relative abundance of LH-PCR fragments were determined using Genemapper V4.0 Software (Applied Biosystems). Samples were standardized and sorted as described in Rees et al. (2004). We cloned and sequenced PCR amplicons generated from a subset of genomic DNA samples using the same primers, but without the fluorescent label. PCR amplicons were cloned into the pGEM-T Vector System (Promega) and taxonomic identity of cloned amplicons was assessed with BLASTn searches.

Soil nutrient availability in the field

During the 2005 and 2006 growing seasons, ion exchange resins (IER) were used to measure the flux of N and P in invaded and uninvaded soils. IER bags were created as described in Hart and Binkley (1984). One IER bag for N and one IER bag for P were placed into each plot during the growing season from mid May 2005–early September 2005, and late April 2006–late July 2006. After incubation the resin bags measuring N flux were extracted in 2 M KCl. The resin bags measuring P flux were extracted in 0.5 M $NaHCO_3$. NH_4^+-N was measured using the phenolate method, NO_3^--N was measured using cadmium column reduction, and PO_4^{3-}-P was measured using the PO_4^{3-}-P

Table 1 Basic characteristics of each study site based on measurements made in 2005. Dominant tree and herbaceous species were based on >25% presence in each 40 × 40-m plot. Mycorrhizal status of the dominant tree species was taken from the literature. Mean density of *Alliaria petiolata* in invaded (*INV*) plots includes both first- and second-year plants (mean ± SE). Mean density of native plants in the INV and non-invaded (*NI*) plots included tree seedlings (<0.5 m in height) and all herbaceous plants ± SE

Site and location	Dominant canopy species	Dominant mycorrhizae	Dominant herbaceous species	Mean density of *A. petiolata* INV plots (plants m^{-2})	Mean % of 1st year *A. petiolata* INV plots	Mean density of native plants INV/NI plots (plants m^{-2})	Annual temperature/ precipitation
Appalachian Trail (AT) Salisbury, Connecticut	*Tsuga canadensis*, *Quercus rubra*, *Populus grandidentata*	Arbuscular/ecto	*Maianthemum canadense*, *Smilacina racemosa*, *Solidago* spp.	177 ± 26	42	43 ± 4/53 ± 10	7°C/1,330 mm[a]
Hammond Woods (HW) Newton, Massachusetts	*Acer plantanoides*, *Q. rubra*	Arbuscular/ecto	*Stylophorum diphyllum*, *Parthenocissus quinquefolia*, *Toxicodendron radicans*	207 ± 36	51	NA/NA	9.6°C/1,681 mm[b]
Norfolk Land Trust (NLT) Norfolk, Connecticut	*Acer saccharum*, *Q. rubra*, *Fraxinus americana*, *A. platanoides*	Arbuscular	*M. canadense*, *Trientalis borealis*, *Impatiens capensis*	204 ± 20	43	22 ± 9/52 ± 10	7°C/1,330 mm[a]
Pine Plantation (PP) Falls Village, Connecticut	*Pinus strobus*	Ectomycorrhizae	*T. borealis*, *Fragaria virginiana*, *Arisaema triphyllum*	223 ± 28	69	27 ± 7/84 ± 15	7°C/1,330 mm[a]
Robin's Swamp (RS) Canaan, Connecticut	*P. strobus*, *Acer rubrum*, *F. americana*	Arbuscular/ecto	*Gallium palustre*, *T. borealis*, *Rubus occidentalis*	172 ± 27	49	25 ± 6/68 ± 6	7°C/1,330 mm[a]

[a] J. Bronson, Great Mountain Forest Corporation, personal communication

[b] NCDC (2005)

method on an autoanalyzer (Lachat Quickchem FIA +8000; Zellweger Analytics, Milwaukee, Wis.). The availability of N or P in the soil was assumed to be the rate at which NH_4^+ plus NO_3^- or PO_4^{3-} accumulated on the resins (μg N or P g^{-1} dry resin day^{-1}).

Soil pH, cation availability and the activity of extracellular phosphatase enzymes were assessed from two soil cores taken from each plot in early May 2006. The top 5 cm of mineral soil was collected using a 5-cm-diameter soil bulk density sampler. One set of samples was used to measure soil pH (1:10 ratio of soil:water, Orion pH meter model 410A; Hendershot et al. 1993a), and cation availability using a $BaCl_2$ extraction (Hendershot et al. 1993b) followed by analysis on a flame atomic absorption spectrometer (Perkin Elmer Analyst 100; Perkin Elmer, Boston, Mass.). The second set of samples was kept at field moisture and analyzed for acid phosphomonoesterase activity, an enzyme responsible for the mineralization of organic P, using the method of Tabatabai and Bremner (1969), with a modified universal buffer at pH 6.5.

Biofumigation experiment

A laboratory experiment was performed to simulate the process of biofumigation. We tested whether the incubation of soils with *A. petiolata* leaves released volatile compounds that affected soil nutrient availability. The experiment consisted of four treatments: incubation with *A. petiolata* leaves, a control with no addition, and incubation with one of two native species. The native species were used to test whether the effect of incubation with *A. petiolata* was significantly different from the effect of incubation with native species. The common, native forb *Impatiens capensis* (jewelweed), and the native tree species, *Acer saccharum* (sugar maple), were chosen to represent the incubated controls as they often co-occur with *A. petiolata* in the field (V. L. Rodgers, personal observation). Fresh *I. capensis*, *A. saccharum*, and first-year rosette *A. petiolata* leaves were collected from three separate locations around the HW site and samples for each species were pooled together. Mineral soil was collected from six uninvaded areas at the AT, NLT, PP, and RS sites in early May 2006 using a 5-cm-diameter soil bulk density sampler to a depth of 15 cm. The samples were sieved through a 2-mm brass mesh and equilibrated in the dark at room temperature for 4 days prior to experimental treatment to allow microbial respiration to stabilize (Degens and Harris 1997).

The soils were incubated in plastic bottles placed within 460-ml Mason jars fitted with a rubber septum. Thirty grams of field moist soil was weighed into each of 192 (4 sites × 6 field replicates × 2 laboratory replicates × 4 treatments) 125-ml Nalgene bottles. Next, two grams (fresh weight) of torn leaf material (2-cm^2 fragments) was placed in the neck of each Nalgene bottle so as to avoid being in direct contact with the soil. All leaves were added to the Nalgene bottles within 4 h of collection and the Mason jars were sealed. After 3 days, the leaves were removed and the jars were left uncapped for 2 h to allow the headspace CO_2 concentration to drop below 2%. Fresh leaves were then collected, added to the jars, and left to incubate for an additional 3 days.

Net N mineralization in the incubated samples was measured over the 6-day period. At the beginning and end of the incubation, pools of NH_4^+ and NO_3^- were extracted from soils (Hofer 2003; Knepel 2003) and NH_4^+-N and NO_3^--N values were measured on an autoanalyzer (Lachat Quickchem FIA +8000; Zellweger Analytics, Milwaukee, Wis.). Net N mineralization was calculated as the difference in total inorganic N concentrations from the incubated and initial soil samples.

Root exudation experiment

We conducted a greenhouse experiment to test the effect of root exudation on soil nutrient availability. We used a randomized block design. Soils grown with *A. petiolata* were compared to control pots with no plants or pots grown with other species of plants. The other plants were used to test whether the effect of root exudation by *A. petiolata* was significantly different from the effect of root exudation by other plants. We examined two native and one agronomic plant species: *Aruncus dioicus* (goat's beard), *Trifolium pratense* (red clover), and *Tricticum aestivum* (wheat). In this experiment first-year rosette plants of *A. petiolata* were used. All plants were grown from seed.

Soil for this experiment was collected in July 2006 from three uninvaded areas within the NLT and PP sites. The soils from both sites were mixed together in equal proportion, and 100 g of soil from this mixture was weighed into each of 80 (5 species treatments × 4 blocks × 4 replicates) square pots (6 cm × 6 cm × 5.5 cm). The pots were then randomly assigned a treatment species, and planted with one recently germinated seedling per pot. The pots were placed under grow lights and watered with 10 ml of distilled water every day. Plants were left to grow for 9 weeks; soil changes have been observed with other plant species after a similar period of time (Troelstra et al. 2001; Klironomos 2002). At the end of the experiment, the roots of all species except *A. dioicus* fully occupied the volume of soil. The entire plant in each pot was then harvested, dried at 60°C, and weighed.

To determine whether root exudation altered nutrient cycling in the soil we measured the concentration of NH_4^+, NO_3^-, and PO_4^{3-} in the soil of each pot at the end of the 8-week period. We also measured soil pH and the activity of extracellular phosphatase enzymes. The concentration of

NH_4^+, NO_3^- and PO_4^{3-} in each sample was analyzed as described above.

Litter decomposition experiment

Two litter decomposition experiments were preformed. The first experiment tested the effect of *Alliaria petiolata* litter on the decomposition of native species litter. The litter bags contained either a single species (herein "monoculture" bags) or a mixture of *A. petiolata* litter placed above the target species (herein "mixture" bags), *Acer saccharum* (sugar maple), *Fraxinus americana* (white ash), and *Quercus rubra* (red oak). This litter was decomposed in an uninvaded stand dominated by the species in the litter bags, located adjacent to the PP site. In November 2004 we collected the leaf litter of three native tree species in three 8 m × 4 m litter traps made of 1-mm-mesh nylon. In July/August 2004 we collected dead *A. petiolata* leaves directly from the stem of senescing, second-year plants. We also collected *A. petiolata* leaves from the rosette stage. The rosette leaves were green at the time of collection, and therefore not technically litter, but we used them to represent the 96–99% seedling mortality that occurs in *A. petiolata* (Cavers et al. 1979; Nuzzo 1993). After collection the litter was air dried for 7 days prior to the construction of the litter bags.

Litter bags were 10 × 10 cm in size, and constructed from 1-mm^2 nylon. Each bag was filled with 2.0 g of leaf litter, separated by a 9 cm × 9 cm square of nylon screening. The monocultures consisted of 1.0 g of litter above and 1.0 g of the same species of litter below the 9 cm × 9 cm screen. The mixtures consisted of 1.0 g of either senesced *A. petiolata* leaves or green rosette leaves above 1.0 g of native litter. There were five types of monoculture bags (i.e., pure *Acer saccharum*, *F. americana*, *Q. rubra*, senesced *Alliaria petiolata* leaves, or green *A. petiolata* rosette leaves), six types of mixture bags (each native species overlaid by either senesced *A. petiolata* leaves, or green *A. petiolata* rosette leaves), three replicate bags per treatment, and five harvest dates for a total of 165 litterbags. The litterbags were placed in the field in late November 2004, and harvested after 2, 4, 6, 9, and 12 months. Following harvest the litterbags were dried at 60°C, weighed to determine mass lost, and then ground, and analyzed for N concentration on an Elantech NC2500 elemental analyzer (CE Elantech, Lakewood, N.J.).

The second litter decomposition experiment tested the effect of invaded soil on the decomposition of native tree litter by placing litterbags in either invaded or uninvaded plots. The second litter experiment was conducted at the AT, NLT, PP, and RS sites. For this experiment, the litter of the same three native tree species was collected in October 2005. We deployed 360 monoculture litterbags (4 sites × 3 species × 2 soil invasion classes × 3 replicate bags per soil invasion class and site × 5 harvest dates). The litter bags were filled with 2.0 g of air-dried leaf litter, placed in the field in November 2005, and harvested after 2, 4, 6, 9, 12 months. After harvesting the litterbags were dried and analyzed for mass loss and N concentration as described above.

Statistical analyses

For all field studies, the field plot was considered to be the statistical replicate. Fungal diversity was analyzed using non-metric multidimensional scaling and multiple response permutation procedures in PC-ORD version 4.34 (1999; MjM Software, Gleneden Beach, Ore.). The number of LH-PCR peaks and peak height were used to calculate Shannon diversity and evenness indices. Variation in the accumulation of inorganic N and P on ion exchange resins, soil pH, base cation concentrations, and extracellular phosphatase activity in the soil were analyzed by two-way ANOVA with invasion type (two levels) and site (five levels) as the main effects. The data for N and P accumulation were averaged by plot across the 2 years of measurement. The biofumigation experiment was analyzed by two-way ANOVA with site (four levels) and treatment (four levels) as the main effects. The effect of root exudation on nutrient availability was analyzed by two-way ANOVA with block (four levels) and treatment species (four levels) as the main effects. The two litter decomposition experiments were analyzed using two-way, repeated-measures ANOVA. In the first experiment, percent mass or N remaining in the litter bag were the dependent variables with the top species in the mixture and the bottom species in the mixture as the independent variables. In the second decomposition experiment, the same dependent variables were modeled as a function of invasion status (i.e. invaded vs. uninvaded) and species of litter. The data were log-transformed when analysis of the residuals deviated from assumptions of normality and homogeneity of variance. Tukey's honestly significant difference range test for post hoc comparisons of mean differences were applied in all post hoc tests to determine significant differences among means (SAS, Cary, N.C.).

Results

Microbial diversity in the field

Fungal community composition was significantly different between invaded and uninvaded soils at the RS site ($P = 0.0012$), but not at the PP ($P = 0.6498$) or NLT sites ($P = 0.1301$) (Fig. 2). Diversity indices, including species richness, Shannon diversity, Shannon evenness, showed no significant differences between invaded and uninvaded plots at any of the sites (data not shown). All cloned and sequenced PCR amplicons matched sequences of fungi in

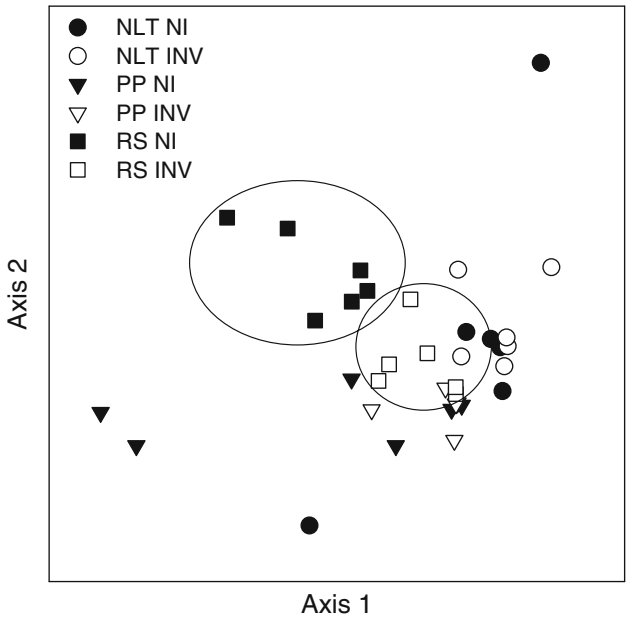

Fig. 2 Non-metric multidimensional scaling plot of polymerase chain reaction (PCR) amplicons from length heterogeneity PCR of the first internal transcribed spacer (ITS1) region of fungal ribosomal RNA. *Rings* indicate samples from the Robin Swamp (RS) site which showed a significant difference in fungal community composition between plots invaded by *A. petiolata* (INV; *open symbols*) and plots not invaded by *A. petiolata* (NI; *filled symbols*). *NLT* Norfolk Land Trust, *PP* Pine Plantation

GenBank in BLAST searches. The cloned PCR amplicons of ITS1 were most similar in sequence identity to ectomycorrhizal basidiomycetes in the Cortinariaceae, Boletaceae, Thelephoraceae, Sclerodermataceae, and Sebacinaceae. Ascomycetes in the Tuberaceae and *Cenococcum* spp. were also present in the clone library.

Soil nutrient availability in the field

The flux of N ($P < 0.05$) and P ($P < 0.001$) to ion exchange resins in 2005 and 2006 was significantly higher in the invaded plots than in the uninvaded plots (Fig. 3). In addition, soil pH ($P < 0.01$), Ca availability ($P < 0.0001$), and Mg availability ($P < 0.0001$) were all significantly higher in the invaded plots than in the uninvaded plots (Fig. 3). The production of extracellular phosphatase enzymes was significantly ($P < 0.05$) lower in the invaded soils than in the uninvaded soils (Fig. 3). Soil moisture ($P = 0.9271$) was not significantly different between adjacent invaded and uninvaded plots (data not shown).

Laboratory experiments

The rate of net mineralization in soils fumigated with *Alliaria petiolata* leaves was not significantly different from those fumigated with *Acer saccharum* or the non-fumigated control soils (Table 2). Fumigation with *I. capensis* ($P < 0.05$), however, had a significant, suppressive effect on net N mineralization as compared to the control soils.

Compared to soils grown with native or agronomic species, the growth of *A. petiolata* rosette roots did not significantly alter the concentration of inorganic N or P in the soil (data not shown). Soil pH ($P < 0.0001$), however, was significantly higher in soils containing *A. petiolata* (5.7 ± 0.03), compared to the control soils (5.4 ± 0.02). The production of extracellular phosphatase enzymes ($P = 0.11$) showed a non-significant, but similar trend to that observed in the field, with soils that contained *A. petiolata* having lower enzyme activity (7.6 ± 1.1 μg g^{-1} dry soil h^{-1}) compared to both the control soils (8.3 ± 1.1 μg g^{-1} dry soil h^{-1}) and the soils containing all of the other species (*A. dioicus* 9.6 ± 1.0; *T. pratense* 9.4 ± 1.0; and *T. aestivum* 9.0 ± 0.7 μg g^{-1} dry soil h^{-1}).

Litter decomposition in the field

In the first experiment, *A. petiolata* litter, both senesced leaves and green rosette leaves, decomposed and released N significantly faster than native tree litter (Fig. 4a, b). When placed above the native litter, *A. petiolata* green rosettes significantly ($P = 0.017$) accelerated the mass loss of native species (Table 3; Fig. 5), and significantly increased ($P = 0.001$) N immobilization in the native species litter (Table 3; Fig. 5). There was no effect of senesced leaves on the decomposition of native tree litter (Fig. 5). In the second litter decomposition experiment, there was no effect of invasion by *A. petiolata* on native tree litter decomposition and percent N remaining over time (data not shown).

Discussion

Invasive plants that alter belowground properties can change soils in a manner that is detrimental to the growth and survivorship of native plants (Callaway and Aschehoug 2000; Stinson et al. 2006). However, the invasive plants themselves may benefit from these changes through a positive feedback (Klironomos 2002). Contrary to our predictions that *A. petiolata* would inhibit microbial activity and decrease soil nutrient availability because of abundant plant secondary compounds, we found that field populations of *A. petiolata* were associated with consistent and significant increases in soil N and P availability, soil pH, and base cation availability (Fig. 3). In a litter decomposition experiment we found that green rosette leaves accelerated the decomposition and N-immobilization phase of native tree species litter (Fig. 5). Short-term laboratory and greenhouse experiments showed that the release of secondary compounds

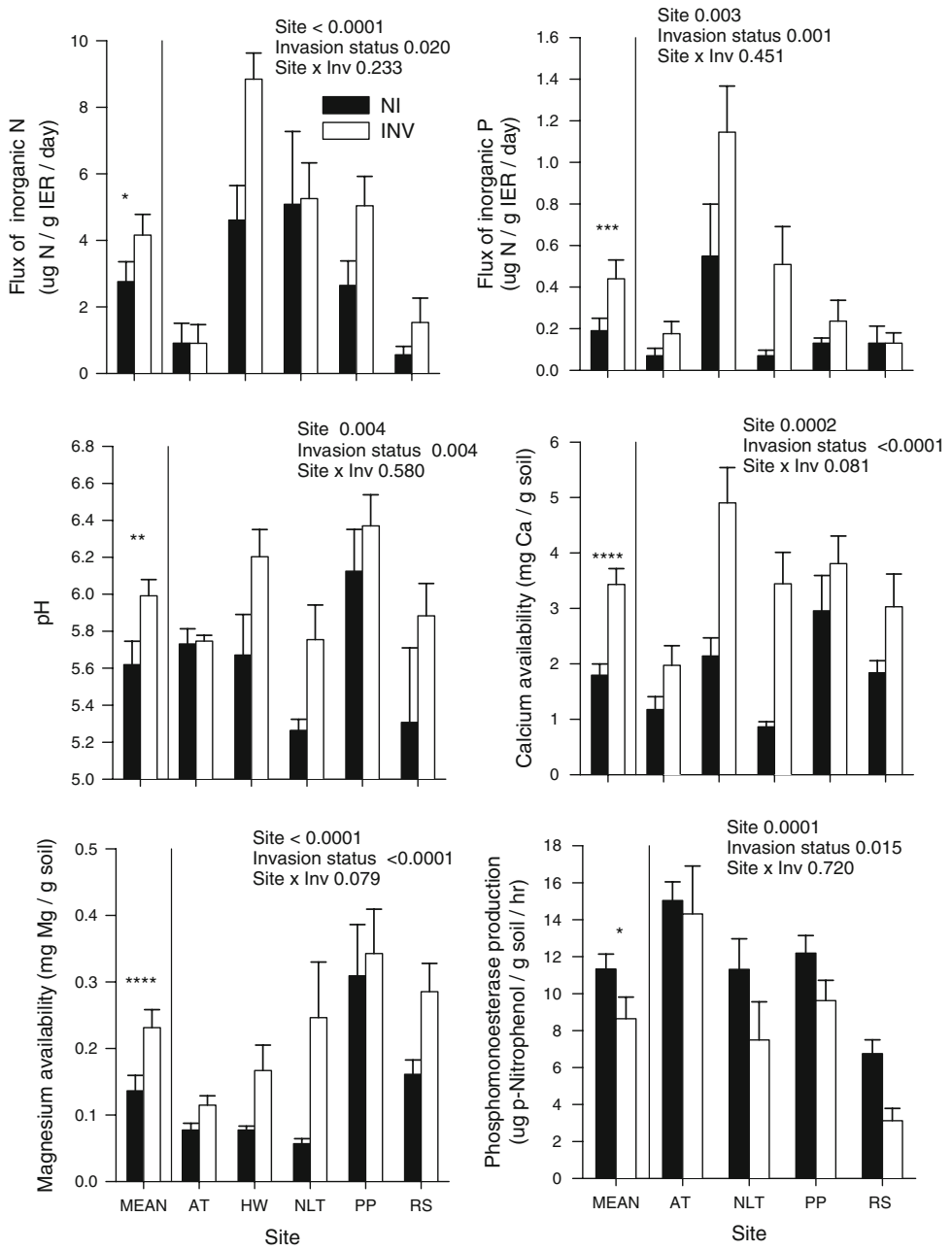

Fig. 3 Average flux of N to ion exchange resins in NI versus INV plots for overall mean and across each of the five sites [Appalachian Trail (*AT*), Hammond Woods (*HW*), RS, PP, NLT] averaged over 2005 and 2006. Flux of N was measured as NO_3^- plus NH_4^+ accumulation on ion exchange resins during the growing season (April–July). Significant differences found for the overall mean ($P < 0.05$) and HW, PP and RS sites ($P < 0.05$). Average flux of P to ion exchange resins in NI versus INV plots for overall mean and across each of the five sites averaged over 2005 and 2006. Flux of P was measured as the accumulation of PO_4^{3-} on ion exchange resins during the growing season (April–July). Significant differences found for the overall mean and AT, HW, NLT, and PP sites ($P < 0.05$). Average pH in NI versus INV plots for overall mean ($P < 0.001$) and across each of the five sites in soils collected in early May 2006. Significant differences found for the overall mean ($P < 0.01$) and HW, and NLT sites ($P < 0.05$). Average Ca availability in NI versus INV plots for overall mean and across each of the five sites in soils collected in early May 2006. Significant differences found for the overall mean ($P < 0.0001$) and AT, HW, NLT, PP and RS sites ($P < 0.05$). Average Mg availability in NI versus INV plots for overall mean and across each of the five sites in soils collected in early May 2006. Significant differences found for the overall mean ($P < 0.0001$) and AT, HW, NLT and RS sites ($P < 0.05$). Average acid phosphomonoesterase production in NI versus INV plots for overall mean and across each of four sites (PP, NLT, RS, AT) in soils collected in early May 2006. Significant differences found for the overall mean ($P < 0.05$) and NLT, PP, and RS sites ($P < 0.05$). *Error bars* represent ± 1 SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

Table 2 Biofumigation experiment mean net mineralization (*net min*) values (±SE) across all four sites for control soils, soils incubated with 2 g fresh weight of *Alliaria petiolata*, *Impatiens capensis*, or *Acer saccharum* in Mason jars ($n = 192$) for a total of 6 days. *Different lowercase letters* indicate significant differences between treatments

Biofumigant	Mean net min (μg N g^{-1} soil day^{-1})
+ Nothing (control)	1.016 ± 0.221 a
+ *A. saccharum*	0.920 ± 0.140 a
+ *A. petiolata*	0.753 ± 0.140 a
+ *I. capensis*	0.694 ± 0.125 b

by *A. petiolata* either via release of volatiles from plant tissues or via exudation from plant roots had little effect on soil properties. Below, we combine the results of this study with recent, related studies to suggest that an invasion by *A. petiolata* into new habitats creates soil conditions that favor its continued proliferation at the expense of native members of the plant community.

Microbial diversity

Recent research demonstrates that invasive plants can inhibit or stimulate soil microbial communities. Invasion by the hybrid cattail, *Typha* × *glauca*, into freshwater wetlands significantly increased soil bacterial diversity, with a positive effect on denitrifier activity (Angeloni et al. 2006). The exotic grasses *Avena barbata* and *Bromus hordeaceous* increased microbial biomass and the abundance of NH_3-oxidizing bacteria in the soil of a California grassland (Hawkes et al. 2005). By contrast, the Australian tree *Melaleuca alternifolia* inhibited microbial colonization of leaf litter in the soils it invaded (Boon and Johnstone 1997).

Invasion by *A. petiolata* has been shown to suppress the growth of arbuscular (Stinson et al. 2006) and ectomycorrhizal fungi (Wolfe et al. 2008). Although *A. petiolata* itself is non-mycorrhizal, 95% of all plant species form symbiotic relationships with mycorrhizal fungi (Smith and Read 1997), and the disruption of this mutualism by *A. petiolata* may decrease the growth and abundance of competing native plants. Indeed, Stinson et al. (2006) found that percent mycorrhizal colonization and the growth rate of native tree seedlings declined significantly when aqueous extracts of *A. petiolata* plants were added to soils. In this study, *A. petiolata* invasion was associated with a significant shift in the fungal community composition at only one of the three sites where we assessed fungal diversity (Fig. 2). Although it is unclear why diversity changed at only one of three sites, at all four of our research sites, Wolfe et al. (2008) found significant declines in the abundance of ectomycorrhizal (ECM) biomass on fine root tips, and Rodgers and Finzi (in review) found that the decline in ECM fine root biomass was correlated with declines in the growth rate of native tree seedlings. Collectively, these studies suggest that invasion by garlic mustard has a large impact on the abundance of mycorrhizal fungi, but that overall diversity of the fungi in the soil is not altered.

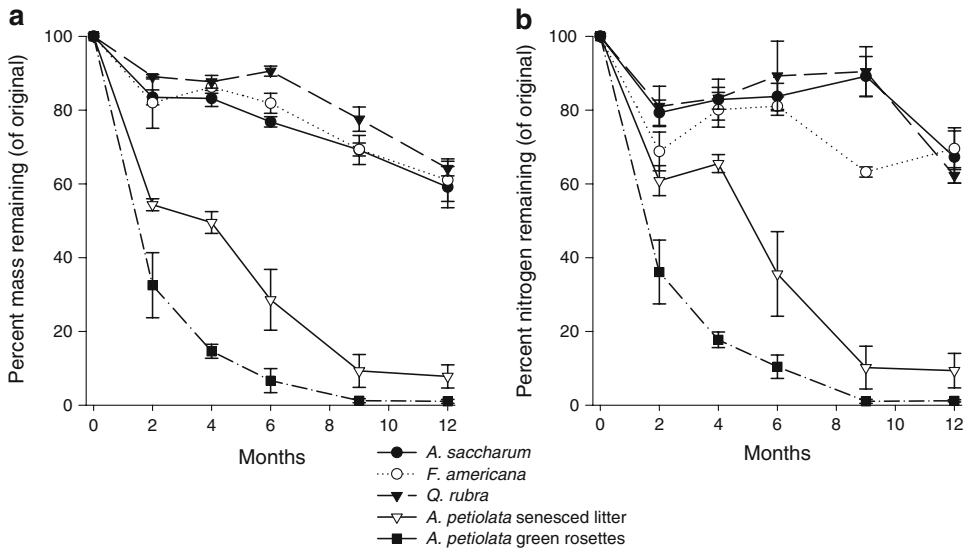

Fig. 4 **a** Average percent mass remaining (of original) over time for *Acer saccharum*, *F. americana*, *Q. rubra*, *Alliaria petiolata* senesced leaf litter, and *A. petiolata* green rosettes as monoculture litter bags in first litter decomposition experiment. *A. petiolata* litter decomposes significantly more quickly than all native tree litter. **b** Average percent N remaining (of original) over time for *Acer saccharum*, *F. americana*, *Q. rubra*, *Alliaria petiolata* senesced leaf litter, and *A. petiolata* green rosettes as monoculture litter bags in first litter decomposition experiment. *A. petiolata* litter loses N significantly more quickly than all native tree litter. *Error bars* represent ±1 SEM

Table 3 Repeated-measures ANOVA for percent mass remaining and percent N remaining of three species of native litter under three top layer species treatments (native, *A. petiolata* senesced, *A. petiolata* green rosette) for first litter decomposition experiment

Source of variation	*df*	Percent mass remaining		Percent N remaining	
		MS	*F*	MS	*F*
Between subjects					
Species (Spp.)	2	972.67	32.24***	172.60	1.08
Top layer (Top)	2	159.04	5.27*	1,815.13	11.37**
Top × Spp.	4	49.05	1.63	383.81	2.40
Error	16	30.17		159.68	
Within subjects					
Month	5	4,988.12	194.74***	1,585.46	19.25***
Month × Spp.	10	63.36	2.47*	119.98	1.46
Month × Top	10	21.16	0.83	599.40	7.28***
Month × Top × Spp.	20	16.21	0.63	134.74	1.64
Error	80	25.61		82.36	

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0001$

Soil nutrient availability

In general, the presence of exotic plant species increases soil nutrient availability (Ehrenfeld 2003). Invaded soils typically have higher concentrations of inorganic N (Mitchell et al. 1997; Kourtev et al. 1999), and higher rates of N mineralization and nitrification (Ehrenfeld et al. 2001; Mack et al. 2001). Despite abundant secondary compounds, invasion by *A. petiolata* was correlated with significantly and consistently greater N and P availability, soil pH, and base cation availability compared to adjacent, uninvaded soils (Fig. 3). There were no significant interactions between site and invasion status, indicating that regardless of the nutrient availability at a given site, the plots invaded by garlic mustard had higher nutrient availability than the uninvaded plots (Fig. 3). This raises the question of whether *A. petiolata* caused the changes observed in the field. If *A. petiolata* only grew in fertile areas, all of the invaded sites would have had higher pH and nutrient availability than all of the non-invaded areas. This was not the case. For every soil variable measured in the field, there were non-invaded areas at one site with higher N and P availability, soil pH, or base cation availability than the invaded areas of another site (Fig. 3). Hence, *A. petiolata* was not restricted to only the most fertile sites. Rather, at just about every site, nutrient availability was greater in the invaded plots compared to the uninvaded plots, regardless of whether the uninvaded plots had high or low inherent nutrient availability. This analysis provides strong circumstantial evidence suggesting that *A. petiolata* caused the changes in nutrient availability and soil pH.

The process by which *A. petiolata* increases the availability of N in the soil remains unclear. Neither incubation with crushed *A. petiolata* leaves in the laboratory (Table 2), nor root exudation by rosettes grown in pots in the greenhouse altered the availability of N, indicating that biofumigation and root exudation had little effect on N cycling, at least over the course of our experiments. Moreover, senesced *A. petiolata* leaves did not alter the rate of litter decomposition or N immobilization in the leaf litter of the native tree species (Fig. 5). However, the presence of green rosette leaves did accelerate the decomposition rate of tree litter (Fig. 5), and mass-balance calculations (not shown) demonstrate that the N mineralized from the rosettes during the 12 months of incubation was more than sufficient to account for the N immobilized in the native species leaf litter in the mixture bags (Fig. 5). In the field, first-year rosette plants are sensitive to summer drought (Byers and Quinn 1998) and approximately 95% die before autumn (Nuzzo 1993), representing a large input of green foliage to the soil. The more rapid decomposition of native species leaf litter in the presence of rosette leaves shows that these high nutrient content, green leaves stimulate decomposition to a greater extent than any negative effect of secondary compounds on the activity of the microbes colonizing and decomposing the leaf litter of the native species. Thus one possible mechanism by which *A. petiolata* may increase N availability is by accelerating the rate of litter decomposition.

Based on the design of our first litter decomposition experiment we cannot separate the stimulating effect of *A. petiolata* rosettes leaves from the potential stimulating effect of any other green leaves on native litter decomposition. Although this limits the interpretation of our data, we suggest that native herbaceous groundcovers do not accumulate green leaves to the extent that *A. petiolata* does in northeastern forests. Therefore the life history strategy of *A. petiolata* (biennial, high density, overwintering rosettes) may explain its ability to change soil characteristics. This suggests that forest communities may also be vulnerable to other invaders with similar demographic characteristics, whether or not they possess secondary compounds.

Invasion by *A. petiolata* also increased soil P availability (Fig. 3). In this study, the increase in soil-P availability was not the result of higher rates of P mineralization from soil organic matter; the activity of extracellular phosphatase enzymes was significantly lower in the plots invaded by *A. petiolata* (Fig. 3). The most likely explanation for the increase in P availability is the increase in soil pH (Fig. 3), a process that decreases sorption of inorganic P by Al and Fe in the soil (Schlesinger 1997). The increase in soil pH in the invaded sites was also correlated with higher base

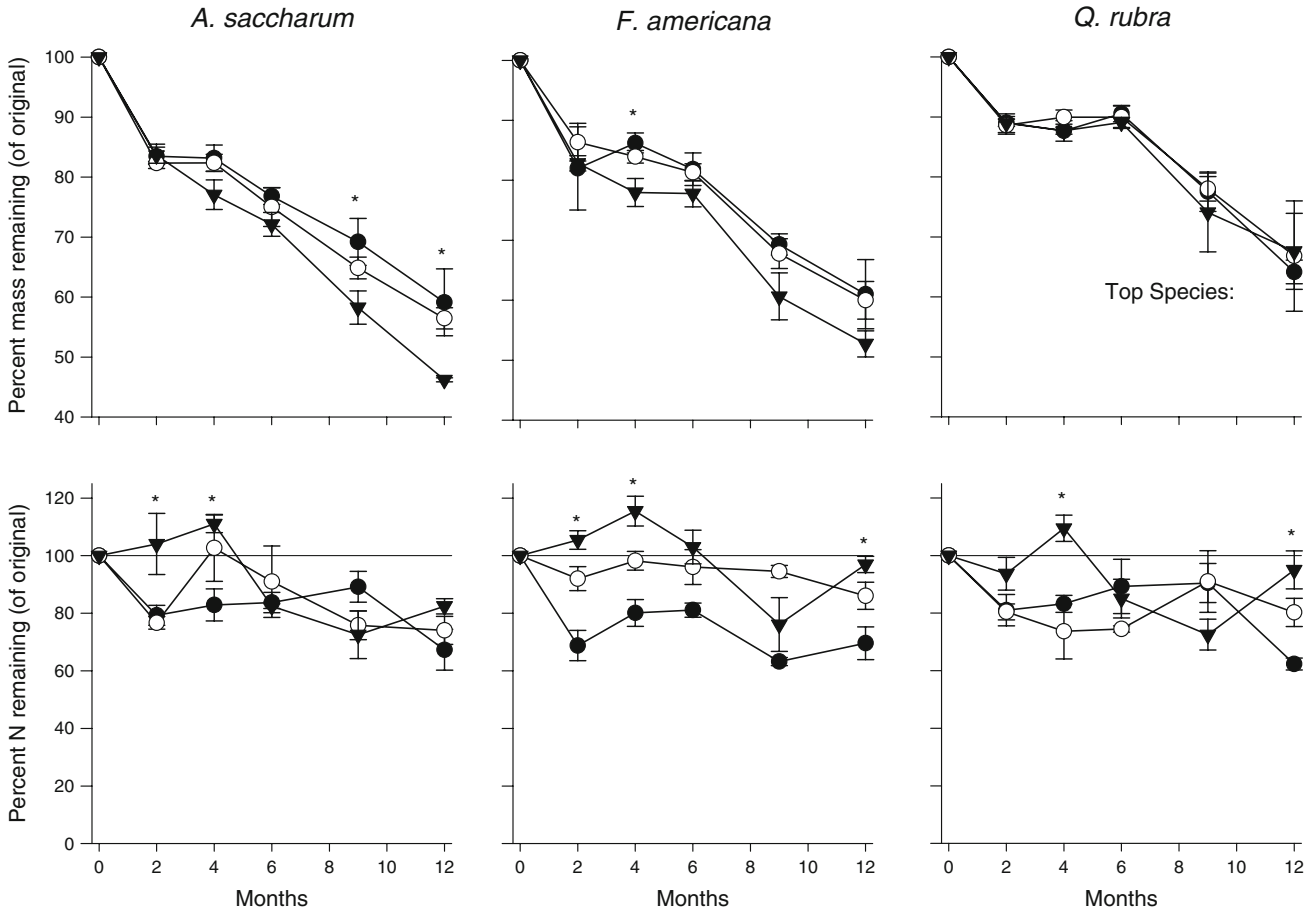

Fig. 5 Average percent mass and N remaining (of original) over time for *Acer saccharum*, *F. americana*, and *Q. rubra* with a top species layer of either the same species (monoculture), *Alliaria petiolata* senesced litter, or *A. petiolata* green rosettes in first litter decomposition experiment. *Error bars* represent ±1 SEM. *Asterisks* indicate time points where *A. petiolata* green rosette treatments significantly differ from monoculture treatments

cation availability (Fig. 3). Given that the increase in soil pH was observed in the root exudation experiment (data not shown), these results suggest that *A. petiolata* raises the pH of soil as a consequence of root exudation, and in turn, creates P- and base-cation-rich soil in which these plants grow.

There are three important limitations to our greenhouse experiments that may have affected our ability to recreate the effects of *A. petiolata* invasion on soil properties observed in the field. First, the experiments were short term (~9 weeks). Thus, it is possible that the subtle changes in pH and the lack of change in N and P availability were due to insufficient time for modification of the soil environment by the roots of *A. petiolata*. It is worth noting, however, that the fine roots of *A. petiolata* completely occupied the volume of soil by the end of the 9 weeks. Second, the root-exudation experiment used rosette plants, which have lower biomass and different growth patterns than second-year flowering plants. After bolting, the second-year plants are typically 1.25 m in height (Cavers et al. 1979) with taproots 5–10 cm in length (V. L. Rodgers, personal observation), and it may be that the second-year plants modify the soil environment to a greater extent than rosettes. Future studies are needed to address the impacts of second-year plants of *A. petiolata* on soil processes. Third, the concentration of secondary compounds within *A. petiolata* tissue has been found to vary with plant age (Cipollini and Gruner 2007) and environmental conditions (Cipollini 2002). Therefore glucosinolate concentrations may have differed between field-grown and greenhouse-grown plants, and even between the five field sites. Estimates for the age of invasion for these sites are currently unknown and therefore the amount of time to allow for the accumulation of cyanide within the soil and the input of fresh rosettes leaves may be a confounding variable. In addition, differences in secondary compound concentrations between plants grown in the field and those grown in the greenhouse may also have affected the amount of cyanide present in the soil, providing an explanation for the discrepancy between the field and greenhouse studies on nutrient availability. Linking the concentration of secondary compounds in plant tissue to specific belowground impacts represents an important area for future research.

Conclusion

The ability of an invasive groundcover, such as *A. petiolata,* to decrease the mycorrhizal colonization of fine roots in native species (Stinson et al. 2006; Wolfe et al. 2008) and simultaneously increase the availability of nutrients in the soil (Fig. 3) represents a novel mechanism by which an invasive plant can impact native forest communities. In related research, Rodgers and Finzi (in review) found that *A. petiolata* grew better in soils in which it previously grew compared to soil previously occupied by native plants, a result corroborated by Klironomos (2002). Given that *A. petiolata* invasion is associated with declines in native plant growth and diversity (McCarthy 1997; Stinson et al. 2007), the results of this study suggest that the changes in nutrient availability putatively caused by *A. petiolata* are detrimental for native plant growth but beneficial for its own growth. Therefore, this invasive groundcover may create a positive feedback between site occupancy and continued proliferation.

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