



## The impact of garlic mustard on sandy forest soils

Sherri J. Morris\*, Dustin L. Herrmann<sup>1</sup>, Jessica McClain, Jaclyn Anderson, Kelly D. McConnaughay

Department of Biology, Bradley University, 1501 W. Bradley Avenue, Peoria, IL 61625, United States

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### ABSTRACT

Garlic mustard (*Alliaria petiolata*), an exotic biennial herb invasive to North American forests, has potential to affect resource availability in invaded soils. Garlic mustard produces a suite of toxic chemicals that impact diversity both above and belowground and thus likely alter ecosystem processes. To examine the effects of garlic mustard on soil biota and ecosystem processes, we sampled soil from invaded and uninvaded stands at a pine plantation on sandy soils in central Illinois. Several of the pine stands were also planted with the N<sub>2</sub>-fixing tree black locust (*Robinia pseudoacacia*). Results indicate that pine soils underlying garlic mustard have higher pH, and exhibit higher rates of N mineralization, relative nitrification and soil respiration compared to soils without garlic mustard. Nitrogen turnover on pine invaded stands is more similar to pine soils with the black locust trees present than to uninvaded pine stands without black locust. Garlic mustard cover was much greater in stands with black locust trees planted, suggesting that garlic mustard may be attracted to high N sites. Catabolic response profiles, which provide a measure of soil microbial function, indicated a shift in substrate use for one of the substrates tested in the presence of garlic mustard. While many of the soil characteristics measured did not differ between invaded and uninvaded stands, the differences with regard to N turnover were striking and will likely have long term effects on soil nutrient status, with potential to feedback to forest health.

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### 1. Introduction

Invasive species are of great ecological and economic concern (Moser et al., 2009). Invasive species threaten native communities by altering community structure and ecosystem function in a manner that can have long term impacts on species diversity and net primary productivity (Mooney and Drake, 1986; Gordon, 1998; Gilliam, 2007). The mechanisms by which species become invasive and alter ecosystem characteristics are related to the physiological and structural characteristics of the invading species and are thus species specific (Ehrenfeld, 2003). Eradication of invasive species requires understanding of the mechanism by which invaders gain advantage in ecosystems. Furthermore, knowledge of the impacts of these species on ecosystem structure and function may be required to restore systems once the invader has been removed (D'Antonio and Meyerson, 2002).

Garlic mustard (*Alliaria petiolata*, Brassicaceae), is a non-mycorrhizal, shade-tolerant herb that invades both disturbed and undisturbed forests in its introduced range (Nuzzo, 1993). Introduced to North America from Europe in the mid 1800s, garlic

mustard had spread to 4 provinces in Canada and 34 states in the US by 2000 (Welk et al., 2002). The models developed by Welk et al. (2002) suggested that garlic mustard had not yet spread to all likely habitats in North America. Garlic mustard proliferates in mesic deciduous forests with partial to full sun, but also inhabits upland areas with well-drained sandy soils (Cavers et al., 1979). Once established, garlic mustard is highly persistent in forests, even remaining through succession and forest transitions (McCarthy, 1997; Meekins and McCarthy, 2001).

Many studies on garlic mustard document belowground impacts following garlic mustard invasion. Studies have reported differences in soil microbial community composition and in nutrient turnover in areas associated with garlic mustard when compared to uninvaded areas (Stinson et al., 2006; Rodgers et al., 2008; Wolfe et al., 2008; Anderson et al., 2010; Lankau, 2010). As with many invasive species these changes to soil microbial populations may persist even following removal of garlic mustard from the affected areas, complicating forest restoration efforts (D'Antonio and Meyerson, 2002).

Garlic mustard, like other plants in the mustard family, has secondary chemicals, primarily glucosinolates, which are toxic to aerobic organisms, including potential herbivores and soil microbes. The cyanide-like moiety that results from glucosinolate degradation in damaged garlic mustard tissues (Cipollini and Gruner, 2007) is likely responsible for its anti-herbivore activity and potential impacts on soils. In its introduced range, the

\* Corresponding author. Tel.: +1 309 677 3016; fax: +1 309 677 3558.

E-mail address: [sjmorris@bradley.edu](mailto:sjmorris@bradley.edu) (S.J. Morris).

<sup>1</sup> Present address: Department of Plant Sciences, University of California, Davis, One Shields Ave., Davis, CA 95616, United States.

glucosinolates in garlic mustard have been held responsible for plant-fungal mutualism disruption and depression of seed germination in native species as demonstrated in laboratory studies (McCarthy, 1997). As a result of microbial transformations, glucosinolates appear to have limited persistence in soils, which may limit their direct allelopathic effects per se (Barto and Cipollini, 2009). However, exposure may result in changes to plant and microbial community structure in a manner that alters nutrient availability and impacts other ecological relationships such as competition and herbivory. In terms of competitive relationships, garlic mustard has been associated with altered nutrient dynamics in its invasive range which may give it advantage over native species. It has been associated with increases in N turnover and key soil-derived plant nutrients, Ca, P, and Mg (Rodgers et al., 2008). Many reports have documented negative effects of garlic mustard invasion on native plant species (McCarthy, 1997; Nuzzo, 1999). These impacts can occur directly as a consequence of suppressed germination of natives or as mediated by impacts on nutrient uptake. In particular, garlic mustard has been shown to suppress arbuscular (Roberts and Anderson, 2001; Stinson et al., 2006) and ectomycorrhizal fungi (Wolfe et al., 2008).

The concerted effects of garlic mustard invasion across soil nutrient classes under the same climatic regime have not been well studied. To uncover this dynamic, we examined multiple proposed effects of garlic mustard in a simple natural system at two fertility levels. This design is unique from those previously field-tested because it holds all factors constant except those affecting soil fertility. Garlic mustard has become widely established on a sandy pine plantation with a low carbon and nitrogen soil matrix in central Illinois. Some stands within the pine plantation are interspersed with the N<sub>2</sub>-fixing tree black locust (*Robinia pseudoacacia* L.) while dominated by pine trees. The presence of black locust has presumably created greater soil fertility in some stands. Black locust has previously been demonstrated to enhance soil N pools and net N mineralization rates in a nutrient poor pine–oak forest (Rice et al., 2004). In general, the study forest has low plant diversity and cover in the understory. These factors may lead to clear signals associated with garlic mustard establishment and persistence.

We hypothesize that garlic mustard invaded soils will have different nutrient conditions and soil microbial activity than uninvaded soils. Specifically, we predict that garlic mustard infestations will be associated with increased carbon and nitrogen turnover, regardless of soil nutrient levels. However, the degree to which differences in soil processes and properties are associated with garlic mustard may vary across a fertility gradient.

## 2. Methods

### 2.1. Study location

The study was located at Sand Ridge State Forest (SRSF), near Manito, Illinois (approximately 3000 ha, 40.41°N latitude, 89.87°W longitude), a glacial floodplain consisting of alluvial deposits from the late Woodfordian era. SRSF was converted from sand prairie and agriculture to pine plantations in the 1930s. Some areas were intermixed with black locust at the time of planting to increase site fertility. For this study, three replicate stands of three stand types were sampled: pine (pine), pine with garlic mustard (pineGM), and pine with garlic mustard and black locust (pineGMBL). No stands could be found with black locust that did not also have garlic mustard. The pines stands studied contained monospecific stands of either red pine (*Pinus resinosa*) or eastern white pine (*Pinus strobes*). Stands were separated by approximately 1 km on average. All soils were of the Bloomfield–Plainfield association and had a loamy sand texture.

### 2.2. Plant survey

To determine if garlic mustard or herbaceous cover varied by stand type and fertility level (i.e., pine, pineGM, pineGMBL), understory plant surveys were conducted in July 2007 in the areas where sampling plots were established. One 25 m transect was established per replicate stand (=3 replicate transects per stand type). Random ¼ m<sup>2</sup> plots were sampled at every meter along the transect at 0–10 m distance perpendicular to the transect. In each plot the % cover by bare ground, garlic mustard, other non garlic mustard weeds, and native herbaceous plants was recorded as coverage class (Class 1: 0–1% cover, 2: 2–5, 3: 6–25, 4: 26–50, 5: 51–75, 6: 76–95, 7: 96–100%) and percent cover per plot was quantified as the mid-point cover for its respective class. Percent cover and relative cover (cover as a percent of total cover) as transect means were analyzed using a one-way ANOVA (SAS 9.1; Proc GLM) on data transformed using the arcsin transformation to meet assumptions of normality, with stand type as the effect.

### 2.3. Soil properties

Soil samples were collected to assess basic properties and microbial activity in garlic mustard invaded vs. non-garlic mustard soil. Within each stand replicate, three 1-m<sup>2</sup> plots were randomly established with at least 1 m distance from the nearest tree. In July 2007, eight composited mineral soil samples from 0 to 10 cm depth were taken with a 2.3 cm diameter soil corer for each plot. Plots in stands with garlic mustard populations were established on currently invaded soils with densities of living first and second year plants not atypical of the stand. Samples were brought to the laboratory, sieved to 4 mm and visible organic (i.e., roots) and inorganic (i.e., rocks) matter was removed. Subsamples were oven-dried at 105 °C to determine moisture content. Soil pH was determined from a 2:1 slurry of 0.01 M CaCl<sub>2</sub> and 15 g air-dried soil. Total soil carbon and nitrogen was determined by sieving a subsample of soil to 2 mm and grinding to pass a 180 µm sieve. Samples were analyzed on a 30 mg subsample on a CE Instruments Flash 1112 Series EA NC Soil Analyzer.

### 2.4. Microbial activity

To determine N mineralization potential, samples were incubated at 70% field capacity at 25 °C for 0, 30, 62 and 120 days. Inorganic N was extracted in 0.5 M K<sub>2</sub>SO<sub>4</sub> and analyzed for NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> on a Lachat Instruments QuikChem® FIA+ 8000 series (Robertson et al., 1999). N mineralization rates were determined as the amount of ammonium plus nitrate produced at the end of the incubation minus that present at the beginning. Relative nitrification represents the amount of nitrate produced during the incubation divided by the N mineralized during that same period (Robertson et al., 1999). Soil respiration was determined by incubating 60 g soil at 70% field capacity at 25 °C. Soil CO<sub>2</sub> flux was measured over a 126-day period using a Li-Cor CO<sub>2</sub> analyzer Model LI-6252 (Paul et al., 2001; Robertson et al., 1999). Basic soil properties were analyzed using one-way ANOVAs with forest type as the effect. Some data required transformation to meet the assumptions of ANOVA, including reciprocal transformation of C:N ratios. The results from C and N mineralization were analyzed using a repeated measures ANOVA (ANOVAR; DataDesk) with the C mineralization data log transformed prior to analysis. The relationship between relative nitrification and pH across treatments was evaluated using pH as a covariate (ANCOVAR; DataDesk). All reported differences are significant at  $p < 0.05$  except where noted with  $n = 9$  for all soil characteristics.

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