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Invasive perennial forb effects on gross soil nitrogen cycling and nitrous oxide fluxes depend on phenology

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Abstract

Invasive plants can increase soil nitrogen (N) pools and accelerate soil N cycling rates, but their effect on gross N cycling and nitrous oxide (N_2O) emissions has rarely been studied. We hypothesized that perennial pepperweed (*Lepidium latifolium*) invasion would increase rates of N cycling and gaseous N loss, thereby depleting ecosystem N and causing a negative feedback on invasion. We measured a suite of gross N cycling rates and net N_2O fluxes in invaded and uninvaded areas of an annual grassland in the Sacramento-San Joaquin River Delta region of northern California. During the growing season, pepperweed-invaded soils had lower microbial biomass N, gross N mineralization, dissimilatory nitrate reduction to ammonium (DNRA), and denitrification-derived net N_2O fluxes ($P < 0.02$ for all). During pepperweed dormancy, gross N mineralization, DNRA, and denitrification-derived net N_2O fluxes were stimulated in pepperweed-invaded plots, presumably by N-rich litter inputs and decreased competition between microbes and plants for N ($P < 0.04$ for all). Soil organic carbon and total N concentrations, which reflect pepperweed effects integrated over longer time scales, were lower in pepperweed-invaded soils ($P < 0.001$ and $P = 0.04$, respectively). Overall, pepperweed invasion had a net negative effect on ecosystem N status, depleting soil total N to potentially cause a negative feedback to invasion in the long term.

Introduction

Exotic plants invade ecosystems in part because of their higher net primary productivity compared to native species (Liao et al. 2008). These invasive plant species often exhibit a fast growth rate on the leaf economics spectrum (Leishman et al. 2007), enabled by greater carbon (C) gain per unit plant nitrogen (N) uptake, or by higher plant N content that supports high levels of N-rich chlorophyll and rubisco needed for photosynthesis (Laungani and Knops 2009). Exotic plant invasions generally lead to accelerated soil N cycling and increases in plant and soil N pools (Liao et al. 2008, Vila et al. 2011, Castro-Diez et al. 2014), creating a positive feedback to invasion (Wedin and Tilman 1990, Allison and Vitousek 2004, Corbin and D'Antonio 2004). These effects are strongest for plants associated with N-fixing bacteria because they increase ecosystem N inputs to enhance the N status

of invaded ecosystems (Liao et al. 2008, Vila et al. 2011, Castro-Diez et al. 2014). If invasive species are not associated with N-fixing bacteria, then accelerated N cycling and related increases in N losses could instead create a negative feedback to the persistence of the invader in the long term. The likelihood of this possibility is poorly understood because invasion effects on ecosystem N outputs, such as gaseous loss via denitrification, are not well characterized (Ehrenfeld 2003).

Non N-fixing invasive plants can increase soil inorganic N availability (Liao et al. 2008, Vila et al. 2011) by stimulating N mineralization (Mack and D'Antonio 2003, Parker and Schimel 2010, Schaeffer et al. 2012). However, this effect is not always observed (Hawkes et al. 2005, Thorpe and Callaway 2011, Laungani and Knops 2012), and few studies measure gross rates of N cycling. Most invasion studies have measured net rates of N mineralization, which are used as indices of plant-available inorganic N after microbial demand is met (Schimel and Bennett 2004). Microbial N immobilization is a strong sink of inorganic N in many ecosystems, and it can compete with plant N uptake (Knops et al. 2002, Laungani and Knops 2012). Changes in microbial N immobilization rates can alter net N mineralization rates even without changes in gross N mineralization rates (Mack and D'Antonio 2003). Therefore, net N mineralization rates can obscure the mechanism leading to invasion effects on soil inorganic N pools. Increased gross N mineralization vs. decreased microbial N immobilization leads to a stimulatory vs. a suppressive effect of invasion on microbial activity.

Invasive plant effects on gross N mineralization vs. N consumption can potentially cascade through microbially mediated N-cycling processes, including those that lead to ecosystem N loss such as denitrification. Gross nitrification rates are, in general, positively related to gross N mineralization, which supplies the ammonium (NH_4^+) substrate for nitrification (Booth et al. 2005). Increases in NO_3^- production via nitrification can lead to corresponding changes in denitrification, which reduces NO_3^- to the major gaseous end products of nitrous oxide (N_2O) and dinitrogen (N_2). This effect could be attenuated if increases in soil NO_3^- also stimulate dissimilatory NO_3^- reduction to NH_4^+ (DNRA), which is often NO_3^- limited in soils (Silver et al. 2001, Yang et al. 2017). Therefore, increases in gross N mineralization could stimulate a linked suite of microbially mediated N-cycling processes resulting in N losses via denitrification, which could be moderated by N retention via DNRA. If plant invasion decreases gross microbial N immobilization, this could potentially lead to lower microbial biomass N (MBN) in the long-term and feed back to suppress gross N mineralization, which is generally positively correlated to MBN (Booth et al. 2005, Yang et al. 2017). Thus, suppression of N immobilization could cascade through the soil N cycle to decrease soil N pools and fluxes.

Lepidium latifolium, commonly known as perennial pepperweed, exhibits many of the classic invasive plant effects on soil N cycling. It has rapidly invaded wetlands, agricultural lands, and grasslands in much of the western

United States since its introduction from Eurasia in the 1930s (Young et al. 1998). It does not associate with N-fixing bacteria, but it has been reported to increase inorganic N pools, as well as rates of net N mineralization and activities of amidohydrolases responsible for microbial N acquisition from organic matter (Blank 2002). This is presumably driven by high foliar N (Runkle 2009), which can lead to N-rich litter inputs to the soil. Higher soil N_2O emissions have also been observed in areas dominated by pepperweed compared to adjacent non-invaded areas, although landscape differences in microtopography in these studies may confound the pepperweed effect (Teh et al. 2011, Yang et al. 2011).

Here, we tested the hypothesis that pepperweed enhancement of soil N pools and fluxes cascades through microbially mediated N-cycling processes in soils. We used ^{15}N pool dilution and tracer techniques to measure a suite of processes that follow the movement of N from organic matter to N_2O emissions, that is, gross N mineralization, gross nitrification, DNRA, and denitrification-derived net N_2O fluxes. We compared soil N pools and fluxes in an annual grassland with and without pepperweed invasion in California. Invasion by perennial pepperweed is a relatively recent phenomenon in the region (<30 yr, Andrew and Ustin 2006) and occurs in a matrix of non-native annual grassland that established as a persistent community well over a century ago (Barry et al. 2006). Changes in soil N cycling with the establishment of annual grasses in native perennial systems have been attributed in part to differences in phenology, namely the shorter growing season of the annual grasses (Adair and Burke 2010, Parker and Schimel 2010). The shallower rooting depth of annual compared to perennial grasses also leads to different soil depth patterns in water and inorganic N uptake (Seabloom et al. 2003). Less work has explored the effects of an invasive perennial in an annual grassland. To account for the role of phenology and rooting depth in driving changes in soil N cycling in response to a shift from annual to perennial plant cover, we sampled in late spring when both plant types were actively growing and also in early fall when pepperweed was dormant and annual plants had not yet germinated following the dry Mediterranean summer at our study site; we also sampled three soil depth increments to 60 cm depth. Overall, we expected to find larger soil inorganic N pools and faster rates of N cycling and greater gaseous N loss with pepperweed invasion.

Methods

Site description

Our study site was located in annual grasslands on Sherman Island in the San-Joaquin-Sacramento River Delta, California (38.04° N, 121.75° W). Grasslands in this region are protected from inundation by a network of levees that maintains a water table ranging from 30 to 70 cm below the surface (Deverel et al. 2007). The regional climate is Mediterranean (hot dry summers and cool wet winters) with a mean annual precipitation of 325 mm

and mean annual temperature of 15.6°C (Teh et al. 2011). Vegetation was largely composed of pepperweed and an exotic annual grass, *Hordeum murinum*, or mouse barley (Sonnentag et al. 2011, Teh et al. 2011). Although mouse barley is not native to this region, it is part of the suite of annual grass species that established and naturalized in the region hundreds of years ago (DiTomaso and Healy 2007); the mouse barley invasion history at our study site is unknown. The soil is classified as a thermic Cumulic Endoaquoll, composed of an oxidized surface layer underlain by a thick organic peat layer (Teh et al. 2011). While mouse barley can be used as forage in grasslands, perennial pepperweed reduces the quantity and nutritional quality of forage (Young et al. 1998).

Experimental design

We established replicate circular plots (1 m radius) spaced 10 m apart along two transects located in adjacent pepperweed-invaded and uninvaded grasslands on a pepperweed invasion front (n = 6 per cover type). The two transects were spaced approximately 80 m apart and were located on the same soil types and slope positions. The uninvaded plots contained no pepperweed and were dominated by mouse barley. The invaded plots contained 25–80% cover by pepperweed interspersed with mouse barley in the understory and had been invaded by pepperweed 3 yr prior to the study.

Pepperweed reaches peak biomass in late spring (Reynolds and Boyer 2010) and senesces in late summer after the fruits mature (Renz and DiTomaso 2004). Thus, we sampled in October 2010 (dormant phase) and June 2011 (active phase). Annual grasses in this region typically reach peak biomass in April–June and die shortly thereafter due to dry summer conditions; their seeds germinate after the first rains of the wet season in October–November (Hatala et al. 2012). When we sampled in October 2010, the first rains of the wet season had occurred but the grasses had not yet germinated; therefore, there was no live aboveground biomass in either invaded or invaded plots at that time. When we sampled in June 2011, the annual grasses were nearing peak biomass; therefore, there was live aboveground biomass in both invaded and uninvaded plots at that time. Pepperweed roots penetrate deep into the soil and to the fringes of water tables (Blank and Young 2004). The water table at the study site fluctuates between 30–70 cm depth (Deverel et al. 2007), so we sampled soil in 20 cm depth intervals to a maximum depth of 60 cm.

We used the stable isotope pool dilution technique to measure gross N mineralization and nitrification rates using $^{15}NH_4^+$ and $^{15}NO_3$ additions, respectively, in laboratory incubations (Kirkham and Bartholomew 1954). We also measured rates of DNRA using $^{15}NO_3^-$ as a tracer (Silver et al. 2001). We expected high rates of gross N cycling in these soils (Teh et al. 2011, Yang et al. 2011), so we performed the incubations over three hours to avoid recycling of the added ^{15}N label (Silver et al. 2001). We minimized methodological artifacts on measured process rates, such as by gently

mixing rather than sieving soils and by adding a small amount of ^{15}N label (~15% of background concentrations), but we note that these rates nonetheless represent potential rather than in situ rates as discussed by Yang et al. (2017). Because many N transformation processes are redox sensitive, we also measured soil oxygen (O_2) concentrations from equilibration chambers buried at 10, 30, and 50 cm depth in the center of each plot (one per depth in each plot) at the time of soil collection for the laboratory incubations (Silver et al. 1999, Yang et al. 2011). We could not locate three of the buried chambers during the dormant phase sampling, but we were able to sample from all chambers during the active phase sampling.

Laboratory experiment

We collected 0–60 cm depth soil cores from three random locations in each replicate plot, separated them by 20 cm depth intervals, and transported them in gas permeable bags at ambient temperature to the lab at UC Berkeley. We removed all coarse roots and litter as well as most fine roots from the soil cores before gently mixing the soil by hand to homogenize. Approximately 300 g of soil was subsampled from each soil core to create a 900 g composite sample for each depth interval per replicate plot (n = 6). The composite soil samples were subsampled into three separate gas permeable bags for three ^{15}N treatments: a control with no ^{15}N addition (30 g oven dry equivalent [ODE]), $^{15}NH_4^+$ addition (30 g ODE), and ^{15} NO_3^- addition (50 g ODE). The control soils were extracted in 2 mol/L KCl to determine the initial NH_4^+ and NO_3^- concentrations assumed for the other treatments. Prior to the initiation of the ^{15}N experiment, we determined the concentration of ^{15}N label solution needed based on background NH_4^+ and NO_3^- and gravimetric soil moisture measured on a fourth subsample from the composite samples. We analyzed NH_4^+ and NO_3^- concentrations in 2 mol/L KCl extractions using colorimetric techniques on a Lachat flow Quik Chem injector autoanalyzer (Lachat Instruments, Milwaukee, Wisconsin, USA). Soil moisture was determined by oven drying 10 g subsamples at 105°C for 24 h; pH was measured on 10 g air-dried soil in 20 mL 1 mol/L KCl. A subsample of each composite sample was also air dried, sieved (to 2 mm), hand picked to remove all fine roots and litter, and ground for C and N analysis on a CE Elantech elemental analyzer (Lakewood, New Jersey).

After adding the appropriate ^{15}N label solution to a bag of soil (Appendix S1: *Methods*), we gently mixed the soil by hand to evenly distribute the ^{15}N label. The soils were immediately transferred to pint sized mason jars and sealed with gas-tight lids fitted with butyl rubber septa for gas sampling. After a 3-h incubation at 25°C, we used a 60-mL polypropylene syringe to extract gas samples from the jar headspace and stored the samples in pre-evacuated glass vials. We analyzed the gas samples for N_2O concentrations on a Shimadzu GC-14A gas chromatograph (Shimadzu Scientific, Columbia, Maryland, USA) equipped with an electron-capture detector. We measured the ^{15}N isotopic value of the N_2O on an IsoPrime 100 continuous flow isotope ratio mass spectrometer (Cheadle Hulme, UK) equipped with a trace gas

analyzer (Cheadle Hulme, UK) and autosampler (Gilson GX271, Middleton, Wisconsin, USA). The standard deviation on four replicate analyses of 40 mL ambient air was 0.0014% ^{15}N-N_2O enrichment.

We calculated total net N_2O fluxes (i.e., from all sources) on a per gram ODE basis by assuming a linear change in N_2O concentrations from ambient air during the 3-h incubation time. The denitrification-derived net N_2O flux was determined from the $^{15}N_2O$ flux from $^{15}NO_3^-$ addition divided by the average ^{15}N enrichment of the NO_3^- pool. The average ^{15}N enrichment of the NO_3^- pool was calculated from the initial and final ^{15}N enrichments of the NO_3^- pool. Due to the rapid conversion of NH_4^+ to NO_3^- via nitrification, the net $^{15}N_2O$ flux from $^{15}NH_4^+$ addition reflects N_2O production from both nitrification and denitrification; therefore, we cannot infer nitrification-derived net N_2O fluxes based on $^{15}N_2O$ fluxes from the $^{15}NH_4^+$ addition.

Immediately after gas sampling, we extracted the entire 30 g ODE soil sample for the $^{15}NH_4^+$ incubations in 2 mol/L KCl. For the $^{15}NO_3^-$ incubations, we extracted a 30 g ODE subsample in 150 mL 2 mol/L KCl. For MBN, we extracted a 10 g ODE subsample in 80 mL 0.5 mol/L K_2SO_4, and placed an additional 10 g ODE subsample in a desiccator for chloroform fumigation for 5 d before K_2SO_4 extraction. We digested the K_2SO_4 extracts using persulfate oxidation. The 2 mol/L KCl extracts and digested K_2SO_4 extracts were analyzed for NH_4^+ and NO_3^- concentrations on the Lachat autoanalyzer. Soil MBN was calculated as the difference between fumigated and unfumigated K_2SO_4 digests without correction (Brookes et al. 1985, Cabrera and Beare 1993). We prepared the 2 mol/L KCl soil extracts for ^{15}N isotopic analysis by acid-trap diffusion (Herman et al. 1995) and analyzed the samples on the IsoPrime 100 interfaced to a Vario Micro Cube elemental analyzer (Elementar, Hanau, Germany). Gross N mineralization, nitrification, NH_4^+ and NO_3^- consumption, and DNRA rates were calculated according to Silver et al. (2001).

Statistical analyses

We used the SYSTAT 13 statistical package (SPSS, Evanston, Illinois, USA) to perform statistical analyses. We performed two-way ANOVAs for soil properties and N cycling rates with cover type, soil depth, and their interactions as factors; these analyses were performed separately for the active and dormant phenological phases. We used Tukey's tests for post-hoc pairwise comparisons. We performed linear regression analyses to characterize relationships between soil properties and N process rates. Unless otherwise noted, regression analyses encompassed all of the data (i.e., cover type and depth data were not separated in the analysis). Data were log-transformed as needed to meet the normality assumptions of ANOVAs and linear regressions. Sample sizes less than $n = 6$ per cover type and soil depth in a given phenological phase reflect negative calculated process rates that were excluded from data analysis. Statistical significance was determined at $P < 0.05$.

Results

Pepperweed invasion altered soil organic matter composition to 60 cm depth (Fig. 1). Soil C:N ratios, SOC, and soil total N were lower in pepperweed-invaded plots compared to uninvaded plots ($P < 0.001$, $P < 0.001$, and $P = 0.04$, respectively). The interaction between cover type and soil depth was significant for soil C:N ratios ($P < 0.001$) but not SOC or soil total N.

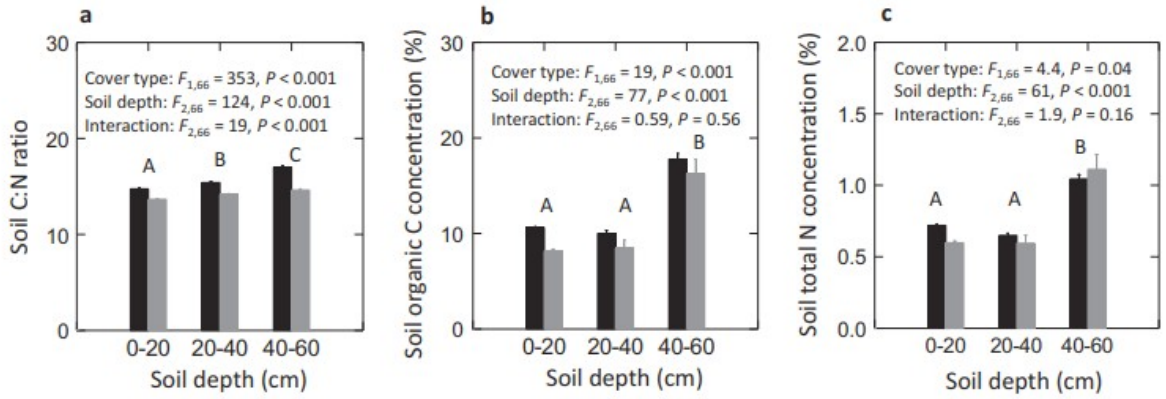

FIG. 1. Soil organic matter composition by cover type and soil depth. Soil (a) carbon to nitrogen (C:N) ratio, (b) organic C concentration, and (c) total N concentration for samples collected from uninvaded (black bars) and pepperweed-invaded (gray bars) plots during dormancy and active growth ($n = 12$). Values are mean + SE. Letters indicate statistically significant differences ($P \leq 0.05$) among soil depths.

Soil moisture, O_2 concentrations, and pH differed significantly between pepperweed-invaded and uninvaded plots, and the cover type effects varied by phenological phase and soil depth (Appendix S1: Table S1). During active growth, soil moisture was lower under pepperweed at 0–40 cm soil depth ($P < 0.001$, Appendix S1: Table S1). During dormancy, cover type did not affect soil moisture. In contrast, soil O_2 concentrations did not differ by cover type during the growing season, but were significantly higher under pepperweed across all soil depths during dormancy ($P = 0.005$, Appendix S1: Table S1). During both phenological phases, soil pH was higher in pepperweed-invaded plots ($P < 0.001$ for both, Appendix S1: Table S1).

When soil N pools differed by cover type, they were lower with pepperweed invasion during active growth and higher with invasion during dormancy (Appendix S1: Fig. S1 and Fig. 2). During active growth, cover type, soil depth, and their interaction significantly affected soil MBN ($P = 0.001$, $P = 0.001$, and $P = 0.002$, respectively; Appendix S1: Fig. S1a), with lower soil MBN in pepperweed-invaded plots at both 0–20 and 20–40 cm depths. Also during the active season, soil NH_4^+ trended slightly lower under pepperweed across all soil depths ($P = 0.08$, Fig. 2a). In contrast, soil NO_3^- was significantly lower in the invaded plots at 0–20 cm depth ($P = 0.05$, Fig. 2c). During dormancy, soil NH_4^+ was significantly higher under pepperweed than mouse barley at 0–20 and 20–40 cm depths ($P < 0.001$, Fig. 2b). In contrast, at this time none of these factors significantly affected soil NO_3^- (Fig. 2d),

and only soil depth significantly affected soil MBN ($P < 0.001$, Appendix S1: Fig. S1b).

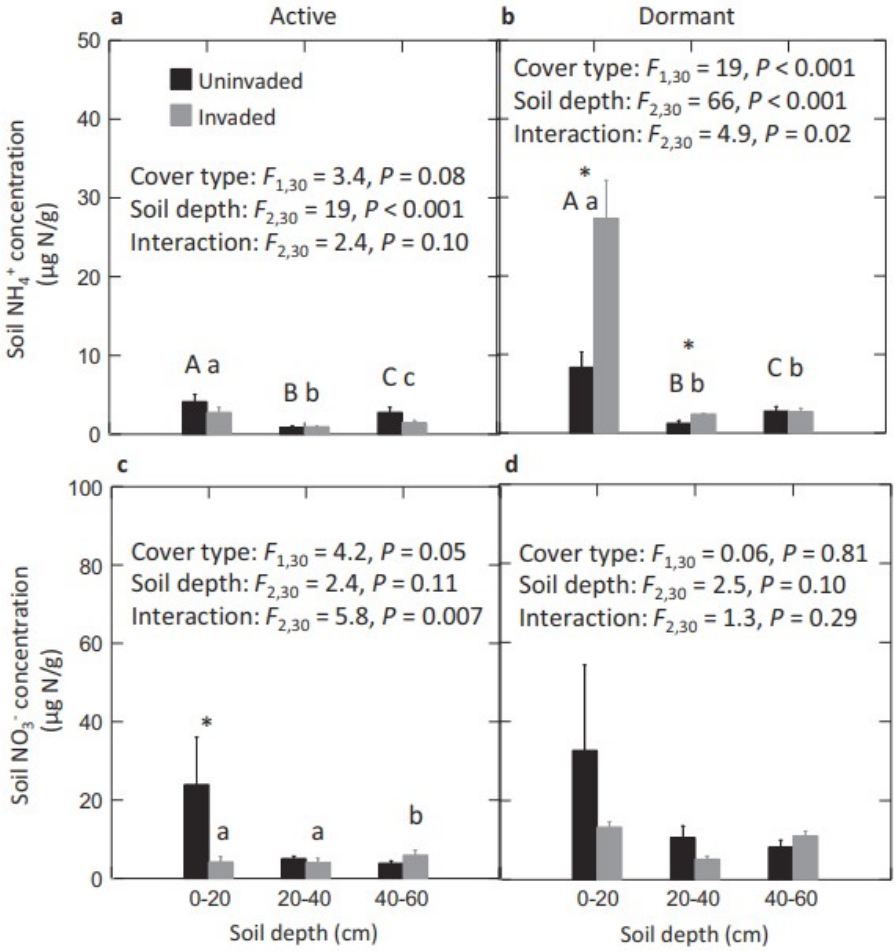

FIG. 2. Soil inorganic N concentrations by cover type, soil depth, and phenological phase. Panels show mean soil ammonium (NH_4^+) during (a) active growth and (b) dormancy and mean soil nitrate (NO_3^-) during (c) active growth and (d) dormancy for uninvaded (black bars) and pepperweed-invaded (gray bars) plots ($n = 6$). Values are mean + SE). Asterisks indicate statistically significant differences ($P \leq 0.05$) between cover types at a given soil depth when there was a significant interaction between cover type and soil depth. Uppercase and lowercase letters indicate statistically significant differences among soil depths for the uninvaded and pepperweed-invaded cover types, respectively.

Soil N cycling rates were also generally lower with pepperweed invasion during the growing season and higher with invasion during dormancy (Fig. 3). Cover type, soil depth, and their interaction had significant effects on gross N mineralization rates in both phenological phases ($P < 0.05$, Fig. 3a, b). During the growing season, gross N mineralization was lower with pepperweed invasion at 0–20 cm depth, but during dormancy, rates were much higher with pepperweed invasion at that depth. Similarly, during active growth, DNRA rates were lower in pepperweed-invaded plots at 0–20 cm depth ($P = 0.001$, Fig. 3e) whereas, during dormancy, they were higher in pepperweed-invaded plots across all soil depths ($P = 0.04$, Fig. 3f). Total net N_2O fluxes were also significantly affected by cover type, soil depth, and their interaction in both phenological phases, with lower fluxes with

pepperweed invasion at 0–20 cm depth during active growth and higher fluxes with pepperweed invasion at 40–60 cm depth during dormancy ($P <$ 0.01, Fig. 4a, b). Denitrification-derived net N_2O fluxes showed similar trends to total net N_2O fluxes, with lower fluxes with pepperweed invasion at 0–20 cm depth during active growth and higher fluxes with pepperweed invasion across all depths during dormancy ($P < 0.02$, Fig. 4c, d). Cover type effects on gross NH_4^+ consumption rates occurred only at 0–20 cm soil depth, with rates lower under pepperweed during the growing season and higher under pepperweed during dormancy ($P = 0.007$ and $P < 0.001$, respectively; Appendix S1: Fig. S2ab). Neither gross nitrification rates nor gross NO_3^- consumption rates differed by cover type during either phenological phase, with soil depth during dormancy as the only significant effect on both rates ($P < 0.001$, Fig. 3c, d and Appendix S1: Fig. S2 cd).

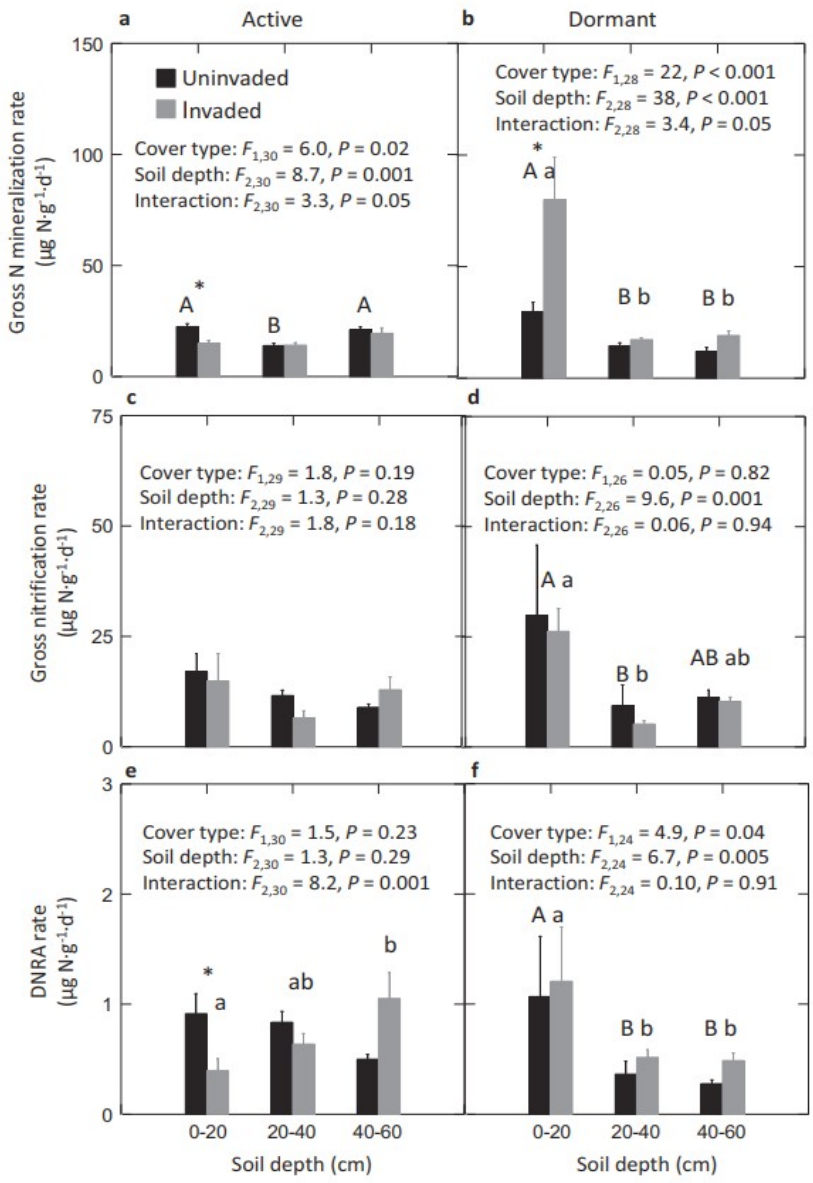

FIG. 3. Soil nitrogen (N) cycling rates by cover type, soil depth, and phenological phase. Panels show mean gross N mineralization rates during (a) active growth and (b) dormancy; gross nitrification rates during (c) active growth and (d) dormancy; and dissimilatory NO_3^- reduction to NH_4^+ (DNRA) rates during (e) active growth and (f) dormancy for uninvaded (black bars) and pepperweed-invaded (gray bars) plots (n = 6). Values are mean and SE. Asterisks indicate statistically significant differences between cover types at a given soil depth when there was a significant interaction between cover type and soil depth. Uppercase and lowercase letters indicate statistically significant differences among soil depths for the uninvaded and pepperweed-invaded cover types, respectively.

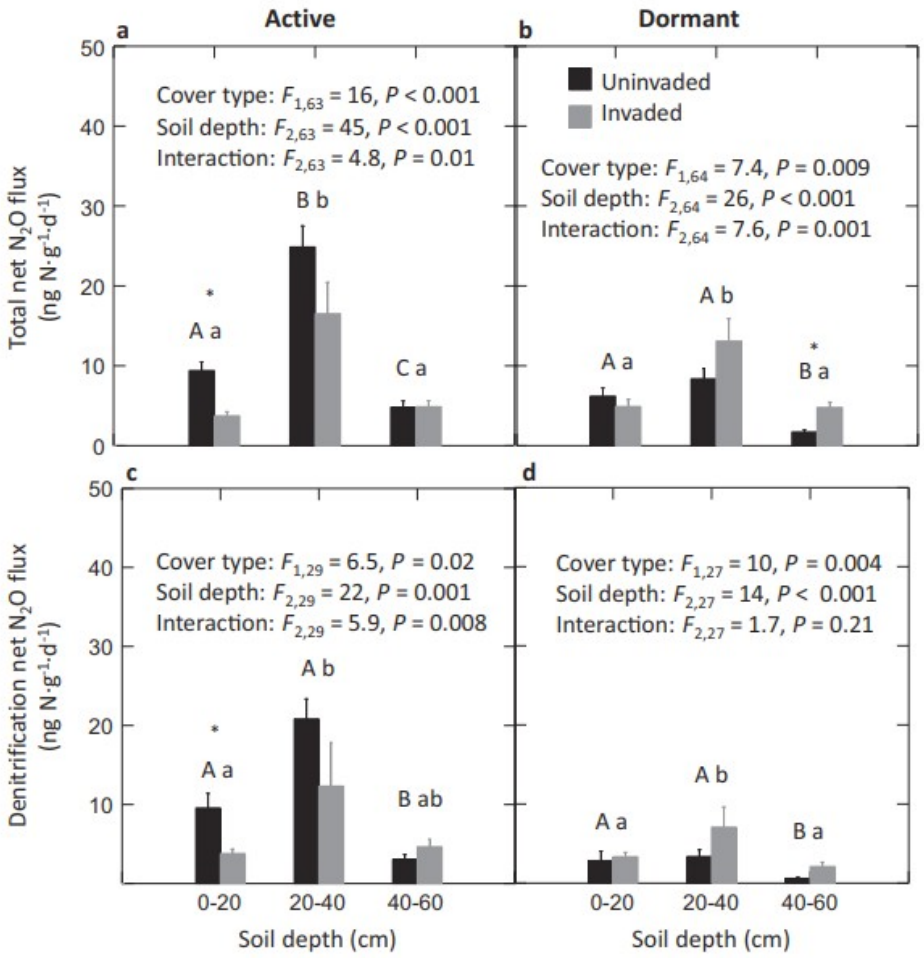

FIG. 4. Net nitrous oxide (N_2O) fluxes by cover type, soil depth, and phenological phase. Panels show total net N_2O fluxes during (a) active growth and (b) dormancy and denitrification-derived net N_2O fluxes during (c) active growth and (d) dormancy for uninvaded (black bars) and pepperweed-invaded (gray bars) plots (n = 6). Values are mean and SE. Asterisks indicate statistically significant differences ($P \leq 0.05$) between cover types at a given soil depth when there was a significant interaction between cover type and soil depth. Uppercase and lowercase letters indicate statistically significant differences among soil depths for the uninvaded and pepperweed-invaded cover types, respectively.

Controls on gross N cycling rates differed by phenological phase. During active growth, gross mineralization rates were positively correlated with both MBN (R^2 = 0.39, n = 36, P < 0.001, Fig. 5a) and SOC concentration (R^2 = 0.39, n = 36, P < 0.001, Fig. 5b) across both cover types and all soil depths. In comparison, during dormancy, soil C:N ratios were the strongest predictor of gross N mineralization rates (R^2 = 0.35, n = 34, P < 0.001, Appendix S1: Fig. S3). Gross nitrification rates across both sampling periods were best predicted by soil NH_4^+ concentrations (R^2 = 0.35, n = 45, P < 0.001), but this relationship was significant only at 0–20 and 40–60 cm depths (Appendix S1: Fig. S4). The relationship between gross nitrification rates and soil NH_4^+ concentrations was stronger during dormancy (R^2 = 0.59, n = 21, P < 0.001) than during active growth (R^2 = 0.10, n = 24, P = 0.14). During dormancy, DNRA rates were positively correlated with soil NO_3^- concentrations (R^2 = 0.47, n = 30, P < 0.001, Appendix S1: Fig. S5).

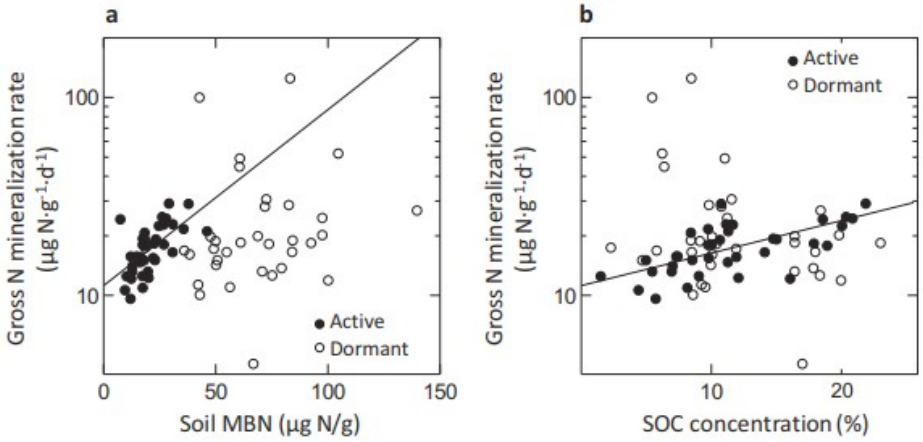

FIG. 5. Gross nitrogen (N) mineralization vs. (a) soil microbial biomass N and (b) soil organic carbon (SOC) concentration by phenological phase across the entire data set. The *y*-axes are shown on $\log_{10}$ scales. Filled circles represent data from the active growth period and open circles represent data from the dormant period. Linear regression lines for the active growth period only are shown. The regression equation for panel a is $\log_{10}(y) = 0.009x + 1.037$ ($R^2 = 0.39$, $n = 36$, $P < 0.001$); and the regression equation for panel b is $\log_{10}(y) = 0.017x + 1.029$ ($R^2 = 0.39$, $n = 36$, $P < 0.001$).

Discussion

Pepperweed invasion affected a suite of surface soil N-cycling processes as we hypothesized, but the direction of the effect depended on phenology instead of consistently enhancing N pools and fluxes as we had expected. During dormancy, gross N mineralization in surface soils (0–20 cm depth) was higher in pepperweed-invaded areas as expected, presumably due to N-rich litter inputs to soil after senescence. In contrast, during the growing season, rates were suppressed by pepperweed. We observed similar phenology-dependent patterns in surface soil inorganic N concentrations, DNRA rates, and denitrification-derived net N_2O fluxes. Rates of DNRA, which competes with denitrification for NO_3^- and supports ecosystem N retention, were orders of magnitude greater than denitrification, yet both processes showed similar patterns across invasion status, phenology, and soil depths. Interestingly, we did not find an invasion effect on gross rates of nitrification, which produces NO_3^- consumed by DNRA and denitrification. Invasive plants can influence the abundance of ammonia-oxidizing microbes responsible for nitrification, which can act as a stronger driver of gross nitrification rates than NH_4^+ supply from mineralization (Hawkes et al. 2005). Overall, we found that the responses of gross N mineralization to invasion can lead to corresponding changes in gaseous N loss from the ecosystem, with lower rates of both fluxes under pepperweed in the active season.

Phenology-dependent patterns in MBN pools suggest that pepperweed may have altered soil N cycling by inhibiting microbial NH_4^+ immobilization. As gross rates of N immobilization are generally positively correlated to MBN (Booth et al. 2005), we can use patterns in MBN as a proxy for patterns in microbial N immobilization. During the growing season, gross N mineralization was positively correlated with MBN, which was lower with

pepperweed invasion. Gross N mineralization may have been limited by the lower microbial biomass or less active microbial communities under pepperweed at that time. Gross NH_4^+ consumption rates, which integrate gross nitrification, NH_4^+ immobilization and other microbial consumption of NH_4^+, were lower in surface soils of pepperweed-invaded compared to uninvaded plots despite no cover type differences in gross nitrification. This provides further evidence of pepperweed inhibition of microbial NH_4^+ immobilization. The lower MBN and gross NH_4^+ consumption rates under pepperweed during the growing season, therefore, suggest that pepperweed was able to outcompete soil microbes for inorganic N to a greater degree than mouse barley, which strongly dominated the uninvaded plots.

We speculate that pepperweed may have inhibited microbial activity via allelopathy. Pepperweed is in the Brassicaceae family, which includes invasive species such as *Alliaria petiolata* (garlic mustard) and common crop species such as *Brassica napus* (rapeseed) known to produce secondary metabolites that suppress the soil microbial community and plant–microbe mutualisms in benefit to plant productivity (Cipollini et al. 2012, Portales-Reyes et al. 2015, Siebers et al. 2018). During dormancy, gross N mineralization was not correlated to MBN, which also did not differ between cover types. We speculate that both a seasonal pulse of litter inputs and relief from plant-derived repression or competition contribute to high dormant season gross N mineralization rate and ammonium pool sizes in surface soils.

Invasion effects on soil organic matter composition reflect longer-term changes in ecosystem N status than instantaneous measurements of soil N pools and fluxes. We observed lower soil C:N ratios under pepperweed, which has also been documented by others (Reynolds and Boyer 2010), but this was not due to increases in soil total N as is more commonly observed in ecosystems invaded by plants associated with N-fixers (Liao et al. 2008). Instead, pepperweed decreased both SOC and soil total N, with a greater depletion of SOC leading to the reduction in soil C:N ratios. The decline in soil total N could reflect a shift of soil N to plant pools caused by lower rates of microbial N immobilization that would make more N available for plant uptake (Laungani and Knops 2012). However, this mechanism is likely more relevant with invasions by woody plants with longer N residence times than forbs or grasses (Laungani and Knops 2009). In this case, the decline in soil total N may reflect a longer-term net loss of ecosystem N under pepperweed that could have a negative feedback on invasion.

Higher soil pH with pepperweed invasion could have contributed to the higher gaseous N loss via denitrification observed during the dormant phase, beyond the direct effect of higher inorganic N availability. Remarkably, after only 3 yr of invasion, soil pH had increased nearly one pH unit under pepperweed. Pepperweed concentrates cations, such as magnesium (Mg^{2+}) and calcium (Ca^{2+}), in soils to cause this increase in pH (Renz and Blank 2004). Plant uptake of NO_3^- and associated root release of hydroxide can

increase soil pH, although this has not been demonstrated for pepperweed. Denitrification rates are well-known to be sensitive to pH, with lower rates at low pH (Simek and Cooper 2002, Cuhel et al. 2010). Consistent with this, a previous study at our site found that denitrifying enzyme activity, a measure of denitrification potential, was positively correlated to soil pH (Yang et al. 2011). Nitrous oxide reduction is suppressed at low pH and thus the N_2O yield of denitrification decreases as pH rises. The general understanding of pH effects on both total denitrification rates and the N_2O yield suggests that N_2 production would be greater in the higher pH soils under pepperweed. Given that elevated soil pH is a common consequence of exotic plant invasion (Ehrenfeld 2003), it is possible that pepperweed increased soil N_2 emissions at this site. Exploring the effects of species invasions on total denitrification should be a priority as new techniques become available for accurately measuring N_2 fluxes (e.g., Yang et al. 2011, 2014).

In conclusion, we found that pepperweed invasion effects on instantaneous soil N pools and fluxes changed with phenology. Over longer time scales, pepperweed invasion is likely to deplete soil total N and potentially cause a negative feedback to invasion. Indeed, a decline in pepperweed stem density has been documented after 15 yr of invasion (Blank and Morgan 2014). Most invasive plant studies measure only a few response variables, focusing on plant and soil properties such as biomass and nutrient pools rather than process rates (Hulme et al. 2013). The few studies that have explored invasion effects on ecosystem N outputs show that invasion increases potential denitrification (Parker and Schimel 2010, Carey et al. 2017) and gaseous N losses (Hickman and Lerdau 2013). Because we measured a suite of soil N pools and fluxes, we were able to link lower gross mineralization rates and smaller inorganic N pools to lower rates of gaseous N loss during a period of plant N uptake; we also measured enhancement of both microbial N-cycling activity and ecosystem N loss during a period of low plant N demand. Our study suggests that measurement of invasion effects during different phenological phases along with ways to integrate effects over longer time scales is needed to assess the net impact of plant invasion on ecosystem N status.

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Data Availability

Data are available from the Illinois Data Bank: https://doi.org/10.13012/b2idb-1324977_v1