# Nitrogen cycling in a pepperweed invaded pasture

Evan F. Portier

## ABSTRACT

Perennial pepperweed (*Lepidium latifolium*) is a pervasive exotic species that has spread throughout the western United States, invading natural and agricultural systems. The objective of this study was to determine if pepperweed impacts gross N cycling rates in an irrigated pasture. I used $^{15}N$ pool dilution and tracer techniques to measure rates of gross N mineralization, gross nitrification, dissimilatory nitrate ($NO_3^-$) reduction to ammonium ($NH_4^+$) (DNRA), and $N_2O$ fluxes. I sampled from replicate plots (n = 6 per cover type) dominated by pepperweed versus dominated by an invasive annual grass (*Hordenum murinem*) with no pepperweed present. Because pepperweed has extensive root systems, I measured gross N cycling rates at three depths (0-20, 20-40, 40-60 cm) to determine pepperweed effects through the soil profile. Soil $NH_4^+$ and $NO_3^-$ concentrations, and gravimetric soil moisture content were also measured at each soil depth. I found that plant cover type affected N cycling differently depending on soil depth, with pepperweed invaded soils exhibiting higher gross mineralization rates ($81.5 \pm 15.3$ µg N $g^{-1}$ $d^{-1}$) and $NH_4^+$ concentrations ($27.3 \pm 4.4$ µg N $g^{-1}$) than grass dominated soils ($30.1 \pm 4.3$ µg N $g^{-1}$ $d^{-1}$ and $8.4 \pm 4.3$ µg N $g^{-1}$ respectively) in surface soil. DNRA, $N_2O$ fluxes, and $NO_3^-$ concentrations were not significantly different between both cover types but were generally highest at the surface depth. These results suggest pepperweed stimulates gross N mineralization to increase soil $NH_4^+$ concentrations for plant uptake without influencing other N cycling processes and pools.

## KEYWORDS

**INTRODUCTION**

The establishment of exotic plant species has profound impacts on ecosystem processes and functioning. Exotic plants displace native vegetation, leading to a reduction in biodiversity, altered trophic dynamics, and increased susceptibility to disturbance (Vitousek, 1990). Coupled with the changes in plant community composition are the physical, chemical, and biological alterations to the belowground environment. For example, the introduction of different root systems, exudation of novel chemicals, and addition of species-specific soil microorganisms all represent various mechanisms by which invasion plants affect the belowground environment (Ehrenfeld, Ravit, & Elgersma, 2005; Wolfe & Klironomos, 2005). Consequently, such changes can influence the nutrient cycling (i.e. the collective processes that transform nutrients into different forms), which is largely mediated by soil microorganisms (Levine et al., 2002)

The effects of exotic plant invasion on soil nitrogen (N) cycling are well recognized (Corbin & D'Antonio, 2004; Ehrenfeld, 2003). N is an essential nutrient to all plants for photosynthesis and other biological processes, and changes in N availability and cycling rates may confer a competitive advantage to invaders (Davis, Grime, & Thompson, 2000). Exotics plants introduce various functional traits that alter soil N concentrations as well as N cycling rates (Ehrenfeld, 2003). The spread of *Myrica faya* introduced biological N-fixing symbionts that subsequently increased soil N concentrations in an otherwise N-limited ecosystem (Vitousek & Walker, 1989). Invasive plants that lack N-fixing symbionts can also raise soil N concentrations through other mechanisms such as increased litterfall (i.e. Aguilera, Alpert, Dukes, & Harrington, 2010). In addition to changing soil N concentrations, invasive plants alter rates of N cycling. *Berberis thunbergii* and *Microstegium vimineum* were shown to increase nitrification rates, a process controlling the availability of inorganic N, despite differences in litter input and chemistry (Ehrenfeld, Kourtev, & Huang, 2001). In contrast, other invasive plants were found to decrease rates of soil N processes upon their displacement of native vegetation (i.e. Evans, Rimer, Sperry, & Belnap, 2001; DeMeester & Richter, 2010). While exotic plant invasion can affect N cycling considerably, the effects of individual species vary and merits greater attention (Ehrenfeld 2003).

Perennial pepperweed (*Lepidium latifolium*) is a pervasive exotic that has rampantly spread throughout the western United States, invading both natural and agricultural systems (Francis & Warwick, 2007). Its rapid proliferation is partially attributed to its high seed

production, rhizomatous growth, and ability to increase nutrient availability in the surrounding environment (Blank & Young, 2004). Pepperweed was found to have high foliar N content, presumably supporting higher photosynthetic rates and enabling invasion (Runkle 2009). In context of N cycling, pepperweed increases microbial enzyme activity associated with N mineralization, the conversion of organic N to inorganic or bioavailable N (Blank, 2002). Elevated inorganic N concentrations were also associated with pepperweed and suggest that stimulated activity of mineralization enzymes potentially results in increased soil N mineralization rates (Blank, 2002). Soil microorganisms associated with N cycling also compete for inorganic N and can potentially stimulate other N cycling processes. Whether pepperweed invasion can affect rates of N cycling in soil remains unclear.

The objectives of this study were to assess the impacts of pepperweed invasion on N cycling in a managed peatland. I hypothesize that pepperweed stimulates gross N mineralization to increase soil $NH_4^+$ concentrations via root exudation and hence gross N mineralization rates and soil $NH_4^+$ concentrations will be elevated throughout the vertical soil profile. I also hypothesize that microbes can successfully compete with pepperweed for $NH_4^+$, resulting in the stimulation of other microbial mediated N processes.  I determined the gross rates of N mineralization and nitrification, processes controlling the bioavailability of soil N, between pepperweed invaded and grass dominated soils. In addition, I determined the rate of soil N retention and loss by the measurement of dissimilatory nitrate ($NO_3^-$) reduction to ammonium ($NH_4^+$; DNRA) and $N_2O$ production respectively. By measuring the above processes simultaneously, I provide an understanding at how gross rates of N processes are influenced by pepperweed invasion. The findings from this study can be used to infer broader ecological consequences and hence directly aid in restoration efforts and management of pepperweed invaded lands.

**METHODS**

**Site Description**

The field site is an upland pasture in the California San-Joaquin-Sacramento Bay Delta, located on Sherman Island (38.04 N, 121.75 W). The pasture is protected from inundation by a

network of levees that maintains a water table ranging from 30 to 70cm below the surface (Deverel, Leighton, & Finlay, 2007). The regional climate is Mediterranean (dry summers and wet winters) with a mean annual precipitation of 325mm and mean annual temperature of $15.6^{o}C$ (Teh et al. 2011). Vegetation at the pasture is largely composed of perennial pepperweed and an invasive annual grass, mouse barley (*Hordenum murinem*; Sonnentag et al. 2011, Teh et al. 2011). The soil is classified as a thermic Cumulic Endoaquoll, composed of an oxidized surface layer followed by a thick, underlying organic peat layer (Teh et al., 2011).

We (Wendy Yang and I) divided the site by dominant plant cover type, pepperweed and grass. The pepperweed cover type was largely characterized by pepperweed over bare soil. The grass cover type was dominated by mouse barley and contained no pepperweed. Within each cover type, we established a 50m transect along which we placed circular replicate plots (n = 6 per transect, 1 m radius) every 10 m.

**Field Sampling**

We (I, Wendy Yang, and several research assistants) sampled in October 2010 (the end of dry season when pepperweed had begun to senesce) and used soil augers to collect soil from 20 cm depth intervals (0-20cm, 20-40cm, and 40-60cm). We transported the soil to U.C. Berkeley in gas permeable bags and stored them at ambient temperature for 2 days before the laboratory experiment (described below). To overcome spatial variability, we collected subreplicate soil samples from three random locations within each replicate plot. At the laboratory, we gently homogenized each subreplicate and combined 300 g from each subreplicate to create a 900 g composite sample. Composite samples were made per depth for each replicate plot.

We also measured soil gas concentrations from soil equilibration chambers at the time of soil sampling. We constructed the equilibration chambers out of polypropylene centrifuge tubes (diameter = 3 cm, length = 15 cm). We drilled a hole on the round end of each tube and inserted a 1/8" Swagelok union. A septum was inserted to the side of the union on the outside portion of the tube. The union was then sealed to the tube with Marine Goop and allowed to cure for 1 day before installation. The chambers were installed in each replicate plot four weeks prior to gas sampling to allow for equilibration with the soil environment. We placed one chamber at each of three soil depths (10, 30, 50 cm) in each plot. The chambers were inserted so that the bottom 5

cm of the chamber filled with soil and the septum port was level with the soil surface. To ensure that the chambers were sealed from the atmosphere, we packed soil around each chamber (Silver, Lugo, & Keller, 1999). We took a 30 mL gas sample from each soil equilibration chamber using a 60 mL polypropylene syringe and immediately analyzed it for $O_2$ concentration on a dissolved $O_2$ meter (Model 52, Yellow Springs Instruments, Yellow Springs, Ohio, USA) with a Clark-type electrode calibrated with atmospheric air.

**Experimental Design**

We conducted $^{15}NH_4^+$ and $^{15}NO_3^-$ isotope pool dilution experiments in the laboratory to measure gross N mineralization and nitrification rates respectively (Silver, Herman, & Firestone, 2001; Templer, Silver, Pett-Ridge, DeAngelis, & Firestone, 2008). To determine gross N mineralization rates, $^{15}NH_4^+$ was added to a soil sample to increase the $^{15}N$ enrichment of the $NH_4^+$ pool. The initial $^{15}N$ enrichment of the $NH_4^+$ pool was calculated from the division of the total concentration of $^{15}N$ (the sum of background $^{15}N$ assuming natural abundance $^{15}N$ enrichment and the added $^{15}N$ label) by the total N concentration ($^{14+15}N$). As soil microorganisms mineralize organic N to $NH_4^+$, the $NH_4^+$ pool becomes isotopically "diluted" by $^{14}NH_4^+$ production (i.e. $^{15}N$ enrichment decreases as mineralization is carried out). After a given incubation time, the change in $^{15}N$ enrichment and total ($^{14+15}N$) $NH_4^+$ concentrations are used to calculate the gross mineralization rate based on the analytical equations derived by Kirkham and Bartholomew (1954). We used the following equations of Kirkham and Bartholomew (1954) in a notation presented by Hart, Stark, Davidson, and Firestone (1994) to measure gross mineralization.

$$\text{m} = \frac{[NH_4^+]_0 - [NH_4^+]_t}{t} * \frac{\log[APE_0 / APE_t]}{\log\left([NH_4^+]_0 / [NH_4^+]_t\right)}$$

where:
m = gross mineralization rate ($\mu$g-N g$^{-1}$ dry weight soil d$^{-1}$)
t = time of incubation (d)
$[NH_4^+]_0$ = initial $^{14+15}NH_4^+$ concentration ($\mu$g-N g$^{-1}$ soil)
$[NH_4^+]_t$ = final $^{14+15}NH_4^+$ concentration ($\mu$g-N g$^{-1}$ soil)
$APE_0$ = initial atom % $^{15}N$ excess of $NH_4^+$ pool

$APE_t$ = final atom % $^{15}N$ excess of $NH_4^+$ pool
  where APE (atom % $^{15}N$) = atom % $^{15}N$ enrichment after the addition of $^{15}NH_4^+$ label
  solution minus the atom % $^{15}N$ at natural abundance


Similarly, gross nitrification rates were determined from the change in the $^{15}N$ enrichment of the $NO_3^-$ pool. $^{15}NO_3^-$ is added to increase the initial $^{15}N$ enrichment of the $NO_3^-$ pool. As soil microorganisms nitrify $NH_4^+$ to $NO_3^-$, the $^{15}N$ enrichment of the $NO_3^-$ pool decreases as a result of $^{14}NO_3^-$ production. The same equation provided above is applicable to gross nitrification rates with the substitution of $NH_4^+$ to $NO_3^-$.

We made six assumptions in order to calculate gross mineralization and nitrification rates from the change in $^{15}N$ enrichment. Violation of the following assumptions can lead to the incorrect calculation of gross rates (Davidson, Hart, Shanks, & Firestone, 1991; Kirkham & Bartholomew, 1954; Murphy et al., 2003).

First, we assumed that gross mineralization and nitrification rates follow zero order kinetics and thus occur at constant rate during the experiment (Kirkham & Bartholomew 1954). Second, we assumed the $^{15}N$ label is not "recycled" back into the product pool during the experiment (Kirkham & Bartholomew 1954). For example, the $^{15}NH_4^+$ label assimilated by microbes is not remineralized back into the $NH_4^+$ pool when measuring gross mineralization rates. Third, microorganisms do not discriminate between $^{15}N$ and $^{14}N$ in consumption processes of the product pool (Kirkham & Bartholomew 1954). While preferential consumption of $^{14}N$ can occur (i.e. Focht 1973), violation of this assumption only causes minor errors if the incubation time is short ( > 1 day) and the level of $^{15}N$ enrichment is minimal (Davidson et al., 1991). Fourth, the $^{15}N$ label was evenly distributed within the sample. We gently homogenized the soil after application of the $^{15}N$ label to reduce the chances of uneven distribution and localization of $^{15}N$ in soil microsites. Fifth, the addition of the $^{15}N$ labeled solution did not stimulate the rates of other N-cycling processes (i.e. N gas production) that are measured in conjunction with gross mineralization and nitrification rates. Other N-cycling processes that use the added $^{15}N$ label as substrate can be enhanced by the addition of large concentrations of $^{15}N$ label and result in incorrect measurements of their rates (Templer et al., 2008). We satisfied this assumption by the minimal application of the $^{15}N$ label. Sixth, we assume the change in the $^{15}N$ enrichment of the product pool is a direct result of $^{14}N$ dilution/production only. In the case of mineralization, the change in $^{15}N$ enrichment of the $NH_4^+$ (product) pool is due solely to the production of $^{14}NH_4^+$.

The organic N (substrate) pool occurs at natural abundance $^{15}$N (atom % = 0.3663) and hence contributes a negligible amount of $^{15}$NH$_4^+$ when mineralized. This assumption is rarely violated as inordinately high gross mineralization and nitrification rates are required to contribute a significant amount of $^{15}$N to the product pool relative to the initial enrichment (~15 atom % $^{15}$N).

We determined gross NH$_4^+$ and NO$_3^-$ consumption rates by treating the $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$ labels as isotopic tracers. We used the equations of Kirkham and Bartholomew (1965) to calculate gross consumption rates from the loss of $^{15}$N from the isotopically labeled pool, which is the substrate pool for consumptive processes. This approach did not distinguish between different consumptive processes. We present the NH$_4^+$ consumption equation in a notation described by Hart et al. (1994):

$$c = m - \frac{[NH_4^+]_t - [NH_4^+]_0}{t}$$

where
c = NH$_4^+$ consumption (μg-N g$^{-1}$ dry weight soil d$^{-1}$)
m = gross mineralization (μg-N g$^{-1}$ dry weight soil d$^{-1}$)
This gross NH$_4^+$ consumption equation was also applied to gross NO$_3^-$ consumption, where we replaced NH$_4^+$ with NO$_3^-$ and gross mineralization (m) with gross nitrification.

In the case of laboratory based pool dilution experiments, we measured individual processes that contribute to gross consumption of NO$_3^-$. We did not measure the constituting processes of gross NH$_4^+$ consumption, which includes nitrification and microbial or abiotic immobilization. The consumption of NO$_3^-$ in closed incubation systems results from three distinct processes: microbial or abiotic immobilization, dissimilatory nitrate reduction to ammonium (DNRA), and denitrification. We measured the latter two processes as they represent N retention and loss pathways respectively.

We used the $^{15}$NO$_3^-$ label to measure rates of DNRA, a microbial mediated process in which NO$_3^-$ is reduced to NH$_4^+$. We calculated DNRA rates as the product of the average NH$_4^+$ concentration and the change in atom % excess of $^{15}$N in the NH$_4^+$ pool, divided by the mean residence time (MRT) of $^{15}$NH$_4^+$ (Silver et al. 2001). We assume a MRT of $^{15}$NH$_4^+$ of 1 day based on previous studies (Silver et al., 2001, Templer et al., 2008). We also divide the above quantity by the average atom % excess of $^{15}$N in the NO$_3^-$ pool because of the variable isotopic composition of the NO$_3^-$ pool during the experiment (Silver et al. 2001)

Denitrification results in the production of $N_2O$ and $N_2$ gas. We measured net $N_2O$ fluxes by the change in $N_2O$ concentrations. We calculated $N_2O$ fluxes on a per g oven-dry equivalent (ODE) basis by assuming a linear change in $N_2O$ concentrations during the incubation time. We did not directly measure denitrification rates as we were unable to detect a change in $^{15}N$ enrichment of the $N_2$ pool within the large background atmospheric $N_2$ pool.

## Laboratory Experiment

Prior to the experiment, we measured background $NH_4^+$ and $NO_3^-$ concentrations (no $^{15}N$ addition) and gravimetric soil moisture to determine the concentration of $^{15}N$ label solution needed. We measured background $NH_4^+$ and $NO_3^-$ concentrations in 2 mol/L (M) KCl extractions. We used a 5:1 ratio of 2M KCl to oven-dry equivalent (ODE) soil subsample (150 mL of 2M KCl to a 30 g ODE subsample). Extracts were placed on an orbital shaker at 200 rpm for 1 hour and filtered through 2M KCl-washed Whatman #1 filters (Whatman, Clifton, New Jersey, USA) to collect the supernatant in specimen cups. We analyzed the filtered extracts for $NH_4^+$ and $NO_3^-$ concentrations using colorimetric techniques on a Lachat flow Quik Chem injector autoanalyzer (Lachat Instruments, Milwaukee, Wisconsin, USA). We measured gravimetric soil moisture by oven-drying 10 g subsamples at $105^oC$ for 24 hours.

We made $^{15}N$ label solutions from 99.7 atom % $^{15}NH_4Cl$ and $K^{15}NO_3$ salts dissolved in deionized water. The target initial $^{15}N$ enrichment was approximately 15 atom % to minimize the stimulation of other N processes while still being able to measure change in the $^{15}N$ enrichment. We made three separate $^{15}N$ label solutions, ranging in concentration because of variable background N concentrations among soils collected from different cover types and soil depths. The average initial $^{15}N$ enrichment was approximately 21.3 atom % for $NH_4^+$ pool and for 15.6 atom % for the $NO_3^-$ pool.

We subsampled 30 ODE g of the composite soil samples into three separate gas permeable bags: no $^{15}N$ addition, $^{15}NH_4^+$ addition, and $^{15}NO_3^-$ addition. The no $^{15}N$ addition soils were extracted in 2M KCl to determine the initial background $NH_4^+$ and $NO_3^-$ concentrations. We added the corresponding $^{15}N$ label solution to the other two bags and gently homogenized the soil 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 hour

incubation, we used a 60 mL polypropylene syringe to extract gas samples from the jar headspace and stored the samples in pre-evacuated vials. We sampled ambient air to characterize the initial gas composition in the jar headspace. After gas sampling, we extracted the soil in 2M KCl to determine $NH_4^+$ and $NO_3^-$ concentrations as previously described.

We used an acid trap diffusion technique to measure the $^{15}N$ isotopic composition of the 2M KCl soil extracts (Brooks, Stark, Mcinteer, & Preston,1989). For $^{15}N$ analysis of the $NH_4^+$ pool, we added magnesium oxide (MgO) to extracts to volatilize $NH_4^+$ to ammonia ($NH_3$). $NH_3$ was subsequently captured on acidified Whatman #3 filter paper disks placed on a wire above the extract. The extracts were tightly sealed in specimen cups after the addition of MgO and stirred daily for 6 days to fully capture the volatilized $NH_4^+$. After the 6 day incubation, the acidified traps were dried in dessicators and folded into tin capsules for $^{15}N$ determination. For $^{15}N$ analysis of the $NO_3^-$ pool, we left the extracts uncovered for 1 day after the 6 day incubation to let any residual $NH_3$ escape. We added Devarda's alloy to reduce $NO_3^-$ to $NH_4^+$ in the extracts, and then the $NO_3^-$ derived $NH_4^+$ was captured on acidified disks as previously described. We analyzed the tin capsules on an Isoprime 100 continuous-flow isotope ratio mass spectrometer (CF-IRMS; Elementar, Manchester, United Kingdom) equipped with a Vario Micro Cube elemental analyzer (Elementar, Manchester, United Kingdom).

We measured $N_2O$ concentrations on a Shimadzu 14-A gas chromatograph (Shimadzu Scientific Inc, Columbia, Maryland, USA) fitted with an electron-capture detector. For $^{15}N$ determination of $N_2O$ concentrations, we analyzed samples on a Isoprime CF-IRMS (Elementar, Manchester, United Kingdom) equipped with a trace gas analyzer (Elementar, Manchester, United Kingdom).

**Statistical analyses**

I used SYSTAT 12 statistical package (SPSS Inc., Evanston, Illinois, USA) to perform statistical analyses to determine statistically significant differences in N processes between cover types and depth and to identify relationships between soil variables and N process rates. I performed separate ANOVAs for gross N mineralization, nitrification, DNRA, and $N_2O$ emission rates using cover type and soil depth as factors.

For regression analysis, the soil variables were assumed to be the explanatory (independent) variables. Unless otherwise noted, regressions analysis included the entire data set (i.e. cover type and depth data were not separated in the analysis). I log-transformed data that was not normally distributed to meet the assumptions of the ANOVAs and linear regressions. I consider p-values less than 0.05 as statistically significant.

## RESULTS

### Gross Mineralization and Nitrification Rates

I found differences in gross N mineralization rates between cover types with significantly higher rates in the pepperweed cover type ($p < 0.05$, Figure 1a). In the case of the pepperweed soils, the average gross N mineralization rate at the surface ($81.5 \pm 15.3$ µg-N g$^{-1}$ d$^{-1}$) was approximately four times greater than rates in the middle (20-40 cm; $17.3 \pm 0.7$ µg-N g$^{-1}$ d$^{-1}$) and lowest (40-60 cm; $19.2 \pm 2.1$ µg-N g$^{-1}$ d$^{-1}$) depths. The highest rates of gross N mineralization occurred at the surface (0-20 cm) compared to the lower depths (20-40 cm, 40-60 cm) for both pepperweed and grass cover types ($p < 0.05$, Figure 1a).

Gross nitrification rates did not differ significantly between cover types (Figure 1b). For surface soils, gross nitrification rates averaged $74.6 \pm 10.6$ µg-N g$^{-1}$ d$^{-1}$ for the pepperweed cover type and $66.9 \pm 34.1$ µg-N g$^{-1}$ d$^{-1}$ for the grass cover type. I found significant trends with depth that were similar between cover types. Gross nitrification rates were highest in the surface soils compared to the lower depths ($p < 0.05$).
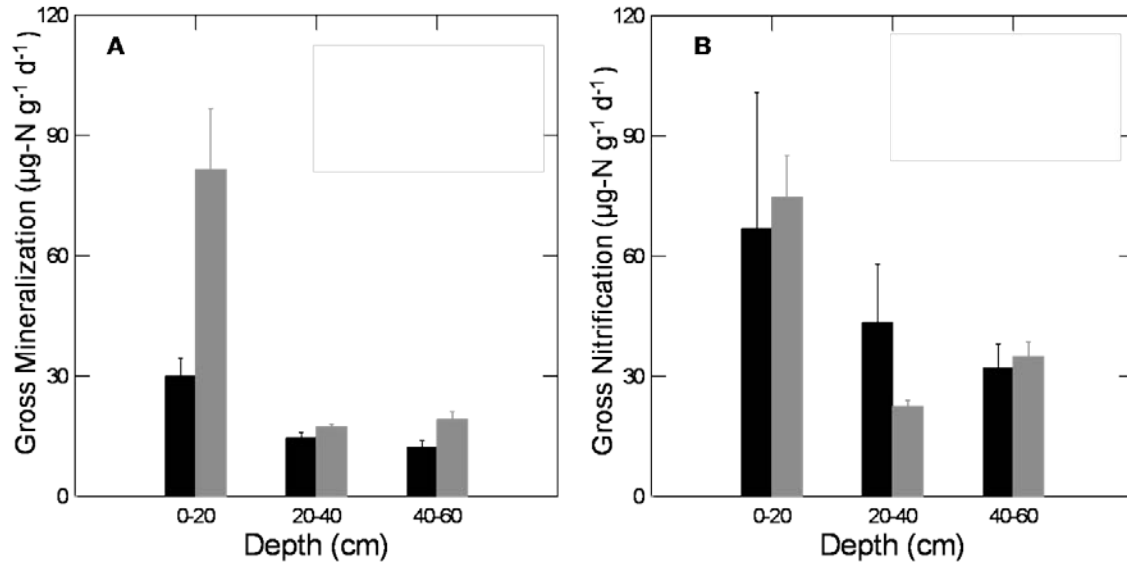
**Figure 1. Gross N mineralization and nitrification rates.** Gross rates of (A) N mineralization and (B) nitrification in grass-dominated soil (black bars) and pepperweed dominated soil (grey bars). Errors bars represent standard errors.

## Consumption Processes

In all cases, gross $NH_4^+$ consumption rates were less than gross production rates (N mineralization; data not shown). In general, I found DNRA rates displayed the same pattern as gross mineralization rates (Figure 2). Differences between cover types were not statistically significant though higher rates occurred in pepperweed- invaded soils at all depths. DNRA rates were also significantly higher at the surface for both cover types ($p < 0.05$), with average surface rates of $0.7 \pm 0.2$ μg-N $g^{-1}$ $d^{-1}$ for pepperweed and $0.7 \pm 0.4$ μg-N $g^{-1}$ $d^{-1}$ for grass cover type.
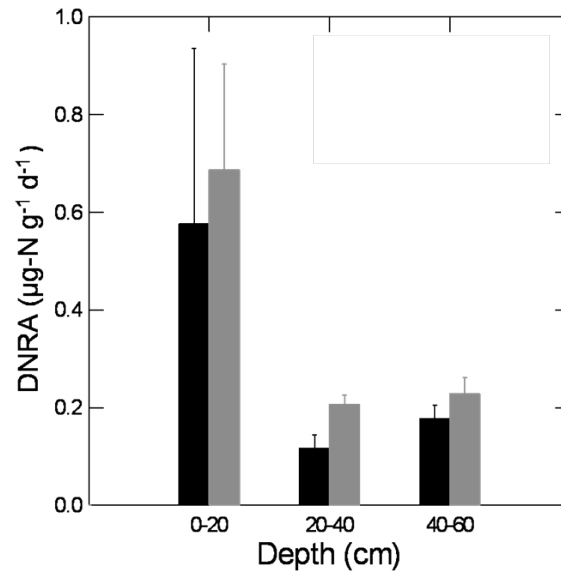
**Figure 2. Dissimilatory nitrate reduction to ammonium (DNRA) rates**. DNRA rates in grass-dominated soil (black bars) and pepperweed dominated soil (grey bars). Errors bars represent standard errors.

The measured $N_2O$ fluxes varied depending on which ^{15}N label was added. For the [15]$NH_4^+$ treatment, I found no significant difference between cover types. I found $N_2O$ fluxes were the highest from the middle depth for both cover types ($p < 0.05$; Figure 3a). The averaged flux was $10.18 \pm 1.99$ ng $g^{-1}$ $d^{-1}$ for the grass cover type and $13.63 \pm 4.30$ ng $g^{-1}$ $d^{-1}$ for the pepperweed cover type at the middle depth. The addition of [15]$NO_3^-$ also prompted changes in $N_2O$ fluxes between the two cover types, where I observed higher $N_2O$ fluxes in the pepperweed cover type ($p < 0.05$, Figure 3b). I found a similar depth trend for the [15]$NO_3^-$ treatment with the highest $N_2O$ flux from the middle depth (Figure 3b). The averaged flux was $5.53 \pm 1.15$ ng $g^{-1}$ $d^{-1}$ for the grass cover type and $7.66 \pm 3.34$ ng $g^{-1}$ $d^{-1}$ for the pepperweed cover type.
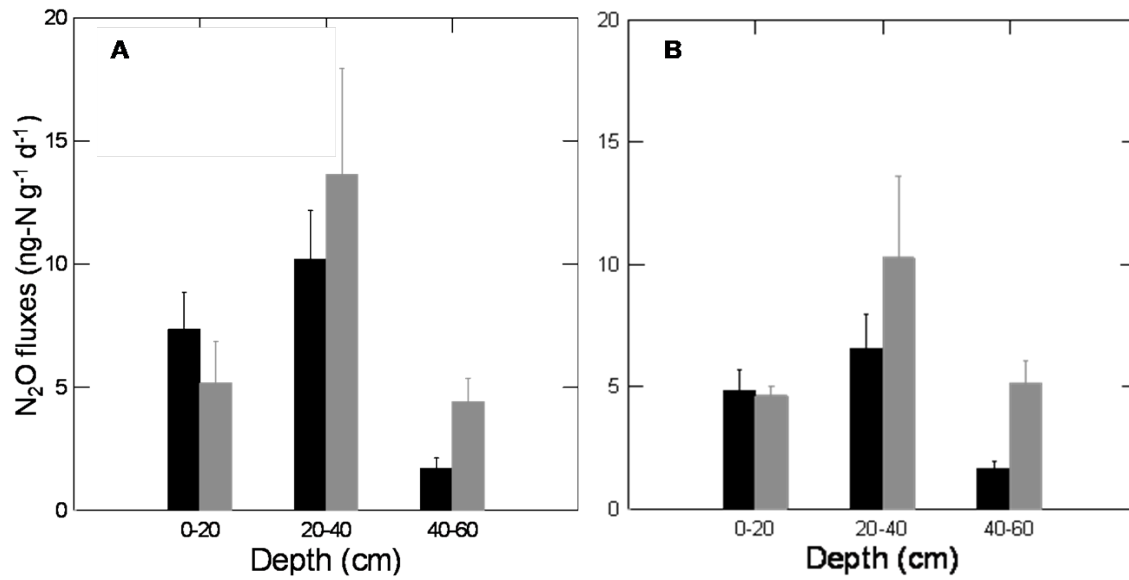
**Figure 3. N$_2$O fluxes.** N$_2$O fluxes from (A) $^{15}$NH$_4^+$ and (B) $^{15}$NO$_3^-$ addition in grass-dominated soil (black bars) and pepperweed dominated soil (grey bars). Errors bars represent standard errors.

## Patterns in Soil Characteristics

I found that NH$_4^+$ concentrations were significantly higher in the pepperweed cover type ($p < 0.05$; Figure 4a). The highest concentrations occurred at the surface, which averaged $8.4 \pm 4.3$ µg g$^{-1}$ for the grass cover type and $27.3 \pm 4.4$ µg g$^{-1}$ for the pepperweed cover type ($p < 0.05$). In contrast, I found NO$_3^-$ concentrations did not differ significantly between cover type or among soil depths (Figure 4b).
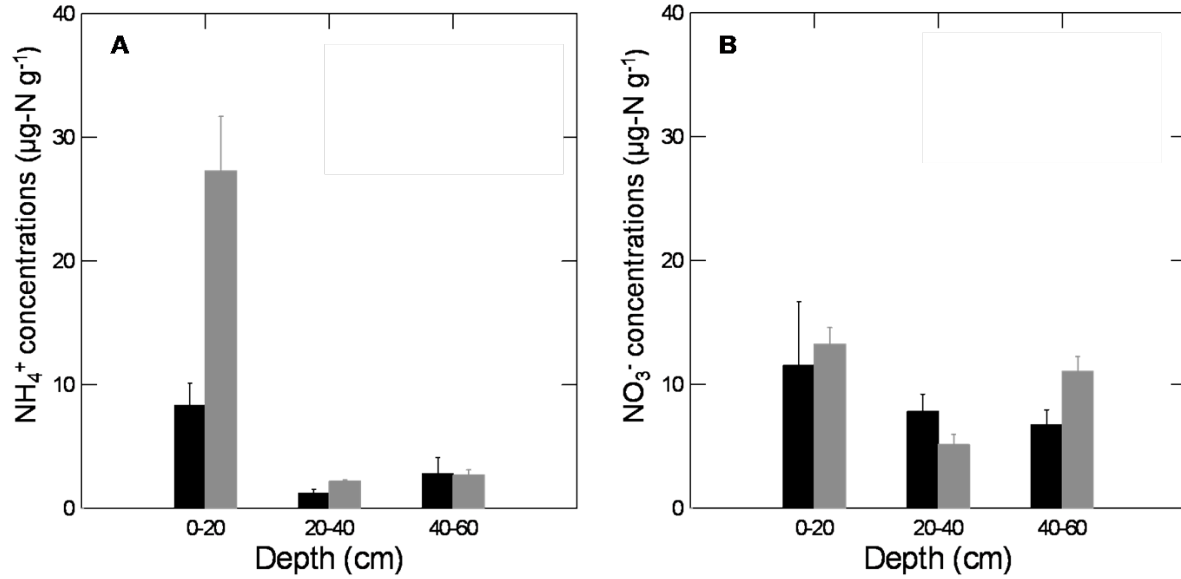
**Figure 4. Inorganic nitrogen concentrations**. $NH_4^+$ (A) and $NO_3^-$ (B) concentrations in grass-dominated soil (black bars) and pepperweed dominated soil (grey bars). Errors bars represent standard errors.

Surface soils for both cover types were the most aerated in terms of soil $O_2$ concentration and soil moisture (Table 1; $p < 0.05$). I found soil $O_2$ concentrations were significantly higher in the pepperweed cover type while soil moisture did not differ between cover types.

**Table 1: Soil characteristics and microbial biomass N**

| Cover type | Depth (cm) | Soil moisture (%) | Soil oxygen (%) |
|---|---|---|---|
| Grass | 0-20 | $29 \pm 2.3$ | $18.9 \pm 0.0$ |
| | 20-40 | $33 \pm 0.8$ | $16.8 \pm 0.3$ |
| | 40-60 | $44 \pm 1.0$ | $15.4 \pm 0.3$ |
| Pepperweed | 0-20 | $24 \pm 0.7$ | $19.6 \pm 0.2$ |
| | 20-40 | $29 \pm 1.1$ | $17.6 \pm 0.4$ |
| | 40-60 | $49 \pm 3.2$ | $15.8 \pm 0.4$ |

*Notes*: Values are reported as mean ± standard deviation.

**Regression Analysis**

I found positive relationships between gross production rates and their respective product pools. Gross mineralization rates were correlated with $NH_4^+$ concentrations ($R^2 = 0.54$) and gross nitrification rates were strongly correlated with $NO_3^-$ concentration ($R^2 = 0.88$). Gross mineralization rates also showed a significant positive correlation with soil $O_2$ concentrations ($P < 0.05$, $R^2 = 0.32$). DNRA rates showed a strong positive correlation with $NO_3^-$ concentrations ($R^2 = 0.69$) and weak positive correlation with soil $O_2$ concentrations ($R^2 = 0.21$).

## DISCUSSION

My results indicated pepperweed invasion influences certain aspects of N cycling in pasture systems. I found that plant cover type and soil depth affected N cycling differently, where pepperweed invaded soils had higher gross mineralization rates and $NH_4^+$ concentrations compared to grass dominated soils. N cycling rates were highest at the surface, illustrating the importance of plant-soil surface interactions. The elevated rates of gross mineralization may provide a mechanism by which pepperweed establishes itself and begins rapid invasion in an ecosystem.

**Gross mineralization and nitrification rates**

Enhanced gross mineralization rates at the surface soils are likely reflective of the different microbial responses to nutrient availability, mainly carbon (C) and N (Chapin, Matson, & Mooney 2002). In general, a relative surplus of either C or N (typically described in the form of a molar C:N ratio) prompts the uptake (immobilization) or release (mineralization) of $NH_4^+$, respectively. A lower C:N ratio in microbial biomass than the surrounding environment promotes mineralization of organic N to $NH_4^+$, as microorganisms are in demand of C rather than N. Such an N-rich (or C-limited) environment may be due to a combination of plant effects and exogenous N-input from fertilizer, groundwater pollution, or animal urine. For example, the elevated surface gross mineralization rates may be potentially fueled by the input of organic material from plant litter. The litter chemistry may be favorable for rapid decomposition in part

because of high foliar N concentrations and specific leaf areas (Allison & Vitousek, 2004). Decomposing organic matter releases dissolved organic N into the soil environment where it can be subsequently undergo N mineralization.

Pepperweed invaded surface soils exhibited gross mineralization rates almost four times compared to grass dominated soils at the surface. Differences in leaf litter chemistry may explain why surface gross mineralization rates in pepperweed invaded soils exceeded the rates observed in grass-dominated soils. Foliar N of pepperweed was measured to be relatively high (Runkle, 2009) and likely favorable to decomposition and mineralization once incorporated into soil. Increased foliar N concentrations may also indicate greater photosynthetic rates and facilitate pepperweed expansion into uninvaded environments. During the senesce period, large amounts of pepperweed litter is then released into the soil and mineralized. However, no physiological traits were measured in this study and the link between N cycling and pepperweed physiology is not well established in the literature.  In addition, Reynolds and Boyer (2010) observed low organic matter input by pepperweed in comparison to native pickleweed (*Sarcocornia pacifica*) in a tidal system, illustrating that pepperweed effects may be ecosystem specific and coupled with environmental conditions. Irrigated pastures in the California Bay Delta tend to have N input from fertilizer and other exogenous sources that may further affect pepperweed growth and litter chemistry. Pepperweed litter likely provides an N rich substrate for elevated rates of gross mineralization because of a suite of factors including high foliar N content and unrestricted growth in an N-rich environment.

Both litter chemistry and litterfall rates are important determinants as to whether invasive plants increase N availability (Aguilera et al., 2010).While high litter input is recognized as a competitive attribute of pepperweed, additional mechanisms may influence gross mineralization rates (Francis & Warwick, 2007). Blank (2002) suggested that pepperweed invasion was potentially mediated through the deposition of labile biomolecules and extracellular enzymes from its roots (collectively termed rhizodeposition), which in turn increased N mineralization activity. My findings do not support this hypothesis as high gross mineralization rates were restricted at surface soils where rhizodeposition effects are relatively minimal (Neumann & Römheld, 2007). These contrasting hypothesizes may also be reflective of various modes of N acquisition that are tied into different phenological stages of pepperweed.

The similar gross nitrification rates for both cover types suggest no direct influence by pepperweed.The background $NH_4^+$ concentrations may be sufficient for soil microbes to carry out nitrification. The additional $NH_4^+$ concentrations in pepperweed invaded soils would thus have minimal effects on gross nitrification rates.

**Consumption Processes**

Gross $NH_4^+$ consumption rates were less than gross mineralization rates in all cases (cover type and depth), hence a net positive N mineralization rate that likely contributes to the standing $NH_4^+$ pool in the soil. The availability of this $NH_4^+$ pool may suggest that plant uptake serves as an important N sink and that preferential uptake of $NH_4^+$ by plants reflects the higher energetic costs associated with $NO_3^-$ uptake (Puri & Ashman, 1999). Furthermore, if litter input lowers soil C:N ratio (described above), gross mineralization will be continued to be favored instead of microbial assimilation of $NH_4^+$.

Among the $NO_3^-$ consumption processes, DNRA rates were highest at the surface soils, further indicating that most microbial mediated N transformations occur at the surface. DNRA rates may be intimately tied with the availability of labile C, suggesting that the surface may retain some pool of labile C (Silver et al., 2001). Net $N_2O$ fluxes were surprisingly low for both cover type and depth, indicating that either denitrification rates are low or gaseous N loss is mainly occurring from the production of $N_2$ gas. Yang, Teh, and Silver (2011) found that $N_2O$ emissions were particularly high in pepperweed-invaded soils at the same study site. The contrasting results may signify phenological effects of pepperweed as my study was conducted in a different season. The effect of cover type on either ^{15}N treatments was not consistent and may indicate that environmental factors influence $N_2O$ production. Overall, consumption processes of $NO_3^-$ were lower compared to gross mineralization and nitrification rates and indicate that N retention and loss processes are less impacted by pepperweed invasion.

**Soil Characteristics**

Higher levels of inorganic N at the surface soil may indicate a balance between biotic and environmental controls. With both high gross production (mineralization and nitrification) and

consumption rates, theory would suggest that standing N pools would be tightly coupled to these processes and concentrations remained low. However, the larger $NH_4^+$ concentrations in pepperweed invaded soils may highlight pepperweed's ability to enhance, either by indirect and direct mechanisms, N concentrations. Although the exact mechanisms by which pepperweed increases N availability is not known, the lack of alleleopathy and fungal N-fixing symbionts (mychorrizae) suggest that pepperweed is superior in its N and other nutrient acquisition to the species its replacing (Young, Turner, & James 1995). As suggested by Blank (2002), increases in inorganic N availability by microbial release of extracellular enzymes or root exudation may prove to be an important invasive mechanism by pepperweed. Blank (2002) also advocated that high surface N availability would not be advantageous to pepperweed because the surface soil dries quickly during the growing season and limits diffusion of N. In contrast, I observed enhanced surface $NH_4^+$ concentrations which may represent a mechanism by which pepperweed expands through lateral rhizomial growth. Pepperweed stands were shown to increase up to 2 m per year by rhizomial growth alone (Orth et al., 2006). The potential stimulation of lateral rhizomial expansion may allow pepperweed to advance the "invasion front" and displace neighboring vegetation.

The exogenous input of $NH_4^+$ and $NO_3^-$ from livestock manure, urine, fertilizer, and N polluted groundwater likely represent important N sources as well. Such sources may stabilize or even enhance inorganic N concentrations despite high rates of N cycling. Because $NO_3^-$ concentrations remained high at the surface and there were no discernible effects by either cover type or depth, exogenous $NO_3^-$ may be a more significant input relative to $NH_4^+$.

**Linking soil characteristics to N dynamics**

The complexity of invasion effects on N cycling is illustrated by the selective enhancement of N processes. However, environmental variables at the ecosystem-scale level are also important drivers for N processes regardless of invasion as well. In the case of $NH_4^+$ and $NO_3^-$ concentrations, my results suggest that the processes that produce them (mineralization and nitrification) remain important controls. In a meta-analysis of the current literature, Booth, Stark, and Rastetter (2003) found that both gross mineralization and nitrification rates across ecosystems positively related to total N concentrations. Additionally, soil oxygen concentrations

remained a good predictor of gross mineralization rates in the California Bay Delta, suggesting aerobic soil conditions promote the mineralization of organic matter.

My results also suggest that $NO_3^-$ concentrations exert a strong control on DNRA rates, agreeing with previous studies (Silver et al., 2001). This suggests that DNRA is a $NO_3^-$-limited process. Surprisingly, rates of DNRA, an anaerobic process, were positively correlated with soil $O_2$ concentrations. Pett-Ridge, Silver, and Firestone (2006) also found higher DNRA rates under aerobic conditions than anaerobic conditions and suggested that facultative microbes (microbes that shift their metabolic requirements) may be dominant among the microbial community carrying out DNRA and more tolerant to variable redox conditions. Indeed, the water table at the study site fluctuates based on land management and seasonal changes, possibly changing redox conditions enough to shift DNRA rates.

## Limitations

The litter chemistry and litterfall rates of both pepperweed and grasses at the field site need to be measured in order to link physiological traits to impacts on N cycling. Additionally, environmental variables such as C:N ratios are also necessary to measure for the same reason. On a broad scale, my results are only applicable to managed ecosystems that may have been susceptible to invasion because of exogenous N input. In N-poor ecosystems, pepperweed might rapidly establish itself through its ability to increase nutrient concentrations relative to native species or other mechanisms. Temporal variation of N fluxes may be associated with the phenological phases of invasive plants and thus there remains a critical need for future studies to account for seasonality.

## Future Directions

To establish a direct link between pepperweed presence and increase gross N mineralization rates, future studies should incorporate a greenhouse component. Furthermore, experiments should determine which mechanism (litter input versus root exudation) is likely responsible for increased rates. If decomposition and N mineralization of pepperweed litter is favorable because of high foliar N content, elevated $CO_2$ emissions may be observed over the

growing season of pepperweed. Thus, it is important for long term monitoring of greenhouse gas fluxes in regions of pepperweed invasion, especially systems where high fluxes already occur such as the California Bay Delta. In addition, I advocate for future studies to incorporate different seasons in order examine how N processes vary on a temporal scale. To evaluate the how climate change and land-use change will affect pepperweed invasion, a thorough biochemical understanding of how pepperweed influences N cycling is also needed, especially in context of belowground plant-soil interactions.

## Broader implications and conclusions

This study is the first comprehensive approach in linking the effects of invasive pepperweed on gross rates of N dynamics. In general, the dominant effect of pepperweed invasion was the alteration of gross mineralization rates and $NH_4^+$ concentrations, both of which may provide a mechanism for its rapid establishment in ecosystems. Retention and loss processes of the N cycle may be less impacted by pepperweed invasion. My results illustrate that while pepperweed invasion does affect N cycling in bulk soil, the mechanism may be a combination of both litter input and root exudation. A greater mechanistic understanding is needed of both the direct and indirect influences of pepperweed on the N cycle as certain environmental variables may preclude any effects by invasion. Additionally, the phenological stages of pepperweed may prompt different changes to the N cycling in the California Bay Delta, demonstrating the complexity of invasion effects and N dynamics.

## ACKNOWLEDGEMENTS

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