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Two invasive plants alter soil microbial community composition in serpentine grasslands

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Abstract

Plant invasions pose a serious threat to native ecosystem structure and function. However, little is known about the potential role that rhizosphere soil microbial communities play in facilitating or resisting the spread of invasive species into native plant communities. The objective of this study was to compare the microbial communities of invasive and native plant rhizospheres in serpentine soils. We compared rhizosphere microbial communities, of two invasive species, *Centaurea solstitialis* (yellow starthistle) and *Aegilops triuncialis* (barb goatgrass), with those of five native species that may be competitively affected by these invasive species in the field (*Lotus wrangelianus*, *Hemizonia congesta, Holocarpha virgata, Plantago erecta*, and *Lasthenia californica*). Phospholipid fatty acid analysis (PLFA) was used to compare the rhizosphere microbial communities of invasive and native plants. Correspondence analyses (CA) of PLFA data indicated that despite yearly variation, both starthistle and goatgrass appear to change microbial communities in areas they invade, and that invaded and native microbial communities significantly differ. Additionally, rhizosphere microbial communities in newly invaded areas are more similar to the original native soil communities than are microbial communities in areas that have been invaded for several years. Compared to native plant rhizospheres, starthistle and goatgrass rhizospheres have higher levels of PLFA biomarkers for sulfate reducing bacteria, and goatgrass rhizospheres have higher fatty acid diversity and higher levels of biomarkers for sulfur-oxidizing bacteria, and arbuscular mycorrhizal fungi. Changes in soil microbial community composition induced by plant invasion may affect native plant fitness and/or ecosystem function.

Abbreviations: AMF – Arbuscular mycorrhizal fungi; PLFA – phospholipid fatty acid; MLG – McLaughlin goatgrass site; MLS – McLaughlin starthistle site; BVG – Bear Valley goatgrass site; CA – correspondence analysis; CCA – canonical correspondence analysis; INVC – invasive patch center samples; INVE – invasive patch edge samples; NAT – native samples

Introduction

To gain insight into the mechanisms and effects of invasion, current research has focused on the 'invasibility' of ecosystems (Lyons and Schwartz 2001; Stohlgren et al. 2001), the 'invasiveness' of certain plants (Rejmanek 2000), and the effects of invasive species on ecosystem functions (Vitousek 1990; D'Antonio and Vitousek 1992; Ehrenfeld 2003). Thus far, only few studies have

focused on how soil microbial communities respond to and/or affect plant invasions (Belnap and Phillips 2001; Klironomous 2002; Kourtev et al. 2002, 2003; Kuske et al. 2002; Duda et al. 2003). This study is the first multi-year field study to examine the impacts of invasive plants on rhizosphere soil community composition at multiple sites and to investigate how these impacts differ along invasion fronts compared to areas invaded for several years.

Plant–soil microbe interactions play a large role in determining plant community structure (Bever 2003) and may in some cases strengthen invasive ability of plants through complex feedback loops (Richardson et al. 2000). Plants provide soil microorganisms with carbon and compete with soil microbes for nutrients. Plants have been shown to structure rhizosphere microbial communities through influences on soil nutrient availability and differential root exudates (Grayston et al. 1996; Westover et al. 1997). Soil bacteria and mycorrhizal fungi, in turn, influence plant community composition and ecosystem function (Allen et al. 1995; West 1996; Requena et al. 1997; van der Heijden et al. 1998). Some soil microorganisms benefit plants by mediating the availability of important plant nutrients (such as N, P, and Fe), forming symbiotic and associative relationships with plants (mycorrhizal fungi, N-fixing bacteria), helping protect against heavy metal toxicity and drought, and producing plant growth-promoting substances such as auxins (Glick 1995). Plant pathogens negatively impact plant fitness and can contribute to plant succession and invasion (Van der Putten et al. 1993; Klironomos 2002; Callaway et al. 2004).

Activities of soil microbial communities govern many ecosystem processes (e.g. N-cycle) and impact plant fitness (e.g. mutualists, plant growth promoting rhizobacteria, pathogens). Thus, shifts in the microbial community composition of soils colonized by invasive plant species may greatly affect ecosystem function and native plant community composition.

We examined the rhizosphere microbial communities of an invasive annual forb, *Centaurea solstitialis* (yellow starthistle) and an invasive annual grass, *Aegilops triuncialis* (barb goatgrass) and compared them with the rhizosphere communities of five native annual forbs (*Lotus wrangelianus*, *Hemizonia congesta*, *Holocarpha virgata*, *Lasthenia californica*, and *Plantago erecta*). These native species were present in higher densities outside goatgrass and starthistle patches than inside the patches, suggesting that these natives may be competitively affected by goatgrass and starthistle in the field.

Yellow starthistle and barb goatgrass are winter annuals native to Eurasia and southern Europe. Starthistle was introduced around 1848 as a seed contaminant of alfalfa (DiTomaso and Gerlach 2000); goatgrass was first introduced in California in the early 1900s (Peters et al. 1996). Both invasive weeds form dense above-ground stands, suggesting that they exert a large impact on the below-ground soil community. We studied starthistle and goatgrass invasion in serpentine grasslands in the Northern California Coast Range where patches of both of these species are expanding their ranges, and the edges of these patches are invasion fronts.

Serpentine soils are highly infertile (low calcium: magnesium ratio, low nitrogen content, high heavy metal content, and low water holding capacity) and tend to host a large number of endemics (Kruckeberg 1984; Huenneke et al. 1990). Many non-native species in California are not successful on serpentine soils, resulting in 'refuges' of native plants in serpentine areas; however, goatgrass and starthistle are capable of invading serpentine soils and, thus, threaten native endemic biodiversity.

We used phospholipid fatty acid (PLFA) analysis to describe rhizosphere soil microbial community composition in this study. The PLFA technique extracts phospholipid fatty acids from living bacterial and fungal cell membranes in the soil and provides a 'microbial community fingerprint' for a sample. PLFA is used to detect differences in total microbial biomass, overall microbial community composition, and lipid biomarkers for specific genera (e.g. *Desulfobacter*, a genus of sulfate reducing bacteria) or classes of organisms (e.g. bacteria, fungi, and protozoa).

We asked the following questions: (1) Are invaded rhizosphere microbial communities different from those of the native plants that the invaders appear to competitively affect? (2) At what spatial scale (within or across sites) are these differences observed? (3) Are there differ-

ences in biomarker fatty acids, total microbial biomass, and/or fatty acid diversity between invasive plant patch centers, invasive plant patch edges, and native plant patches?

Materials and methods

Field site descriptions

The McLaughlin Natural Reserve and Bear Valley are located in the Northern California Coast Range. Three serpentine grassland sites were sampled in this study: McLaughlin goatgrass (MLG), McLaughlin starthistle (MLS), and Bear Valley goatgrass (BVG). 'Sub-sites' were sampled within each of these sites; each sub-site contains an invasive plant patch (comprised primarily of either goatgrass or starthistle at approximately 50% or greater cover) surrounded by a primarily native plant community (Figure 1). Soil was sampled from the center and edge of invasive plant patches as well as external to the patch in the native plant community.

MLG contains sub-sites A, B, and C; MLS contains sub-sites D and E; BVG contains sub-sites X and Y. Within the MLG and MLS sites, invasive plant patches have changed their spatial distribution from 2001 to 2003. MLS starthistle patches in sub-sites D and E expanded their radii by 1.0 and 5.0 m, respectively; the original radii of these starthistle patches were approximately 5 m each. MLG goatgrass patches A, B, and C expanded their radii by 11.5, 5.5, and 5.0 m, respectively, and their original radii were approximately 15, 15, and 20 m, respectively. Thus, we conclude that their distribution is not confined to a certain range within these sites, the invasions are actively progressing, and the edges of these patches can be considered invasion fronts.

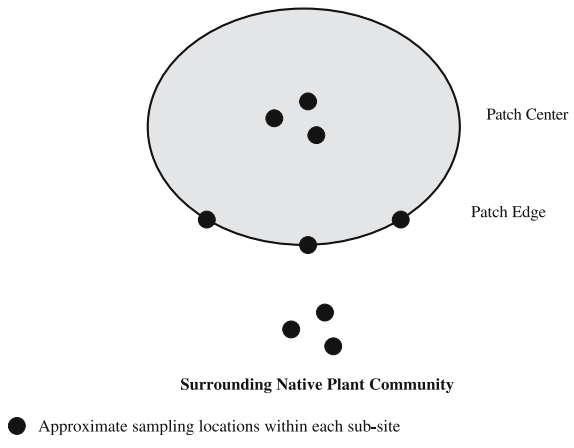

Figure 1. Sub-site schematic diagram and sampling design.

An initial field study at the MLS and MLG sites found that *L. wrangelianus*, *H. congesta*, and *H. virgata* were present in higher densities outside than inside starthistle patches (data not shown). Additionally, *L. californica* and *P. erecta* were present in much higher densities outside of goatgrass patches. Thus, the rhizosphere microbial communities associated with starthistle and goatgrass were compared to those of *L. wrangelianus*, *H. congesta*, and *H. virgata* and *L. californica* and *P. erecta*, respectively.

The climate at the field sites is Mediterranean, characterized by wet winters and hot, dry summers. All plants in this study are winter annual plants, germinating in the winter, and flowering in the spring to summer. Goatgrass, *L. californica*, *P. erecta*, and *L. wrangelianus* are all drought 'avoiders', senescing before the summer drought; whereas starthistle, *H. congesta*, and *H. virgata* are drought 'tolerators', accessing water in the summer with deep tap roots.

Sampling methods

Samples were collected from the McLaughlin Reserve in April of 2001 and 2002 and from Bear Valley in April 2002 (Figure 1). Plant-impacted soil samples were collected by centering a one-inch diameter soil corer around the desired plant and coring to a depth of approximately 10 cm. Each sampled plant was surrounded by plants of the same species as nearest neighbors, and most of these plants grow in relatively high densities (especially goatgrass, starthistle, *L. californica*, and *P. erecta*) and, thus, likely have a large impact on the surrounding soil. Therefore, the soil from each core is *operationally* defined as 'rhizosphere soil' even though this is not the common definition of rhizosphere soil. Goatgrass and *L. wrangelianus* have lateral root distributions; whereas, the rest of the plants

studied are tap rooted. At the time of sampling (April), most of the plant roots contained in the soil samples were less than 2 mm in diameter, with the exception of some of the starthistle, *H. congesta*, and *H. virgata* tap roots. Roots were removed from the soil with tweezers prior to analysis for microbial community composition. Three replicate invasive plant rhizosphere soil samples were taken from the center and edge of McLaughlin invasive plant patches in 2001, and only from the center of patches in 2002 (Figure 1). To minimize differences in soil properties between the soil samples, three replicate native plant rhizosphere samples were collected within 7 m of invasive patch edges at each site (Figure 1). Each soil core was homogenized in separate plastic Ziploc bags in the field, and samples were stored at −20 °C until analysis.

Determination of microbial community composition

Microbial community composition was measured using phospholipid fatty acid (PLFA) analysis described previously (Bossio and Scow 1998; Macalady et al. 2000). Lipids were extracted from 8 g of each soil sample using a one-phase chloroform/methanol/phosphate buffer solvent. Phospholipids were separated from non-polar lipids and converted to fatty acid methyl esters (FAMEs) before analysis on a Hewlett Packard 6890 GC, using a 25 m Ultra 2 (5% phenyl)-methylpolysiloxane column (J&W Scientific). Peaks were identified using bacterial FAME standards and MIDI peak identification software (MIDI, Inc., Newark, Delaware). Previous work in our laboratory used capillary gas chromatography–mass spectrometry (GC-MS) to confirm peak identifications and double bond positions in monounsaturated fatty acids by analysis of dimethyldisulfide adducts (Macalady et al. 2000).

Statistical analyses

Correspondence analysis (CA) and canonical correspondence analysis (CCA) were performed using CANOCO software (Microcomputer Power, Ithaca, New York). Nanomoles of individual fatty acids per gram of soil were used in the analyses. CA is a multivariate statistical method that allows for comparison of fatty acid 'fingerprints' between samples. A CA was performed on the overall PLFA dataset containing data from all soil samples analyzed. Additional individual CAs were performed on PLFA data from each sub-site. CCAs were also performed on the overall PLFA dataset and PLFA data from each sub-site in order to examine the significance of categorical environmental variables (e.g. plant type: invasive versus native) in explaining the ordination of samples based on their fatty acid content. CAs and CCAs were performed using fatty acids that were present in at least 25% of the samples, a strategy that maximized the number of fatty acids used in the analysis without introducing GC detection limit error and avoided the unduly large influence that rare fatty acids can have on unimodal analyses such as CA and CCA (ter Braak and Šmilauer, 1998).

We tested for the significance of differences in mean individual biomarker fatty acid abundance, total microbial biomass, and fatty acid diversity at the centers of invasive patches, invasive edges and in native species patches using restricted maximum likelihood (REML) for a total of 11 comparisons. We selected biomarker fatty acids present in greater than 25% of the samples for analysis. However, total microbial biomass and fatty acid diversity were calculated based on all fatty acids, even those present in less than 25% of the samples. We used REML rather than ANOVA because our sampling design was unbalanced. We performed these analyses in Genstat (Version 6.1, VSN International Ltd, Hemel Hempstead, UK). Multiple comparisons of this kind result in inflated experiment-wise Type I error rate. We applied a sequential Bonferroni correction to the significance level ($P < 0.05$) to obtain corrected significance levels. However, the Bonferroni correction is conservative, so we interpreted uncorrected $P \leq 0.05$ to provide cautious support for differences in fatty acid biomarkers, and P values less than the Bonferroni corrected significance level to provide strong support for differences in fatty acid biomarkers.

Results

Comparison of microbial community composition across all Sites (MLG, MLS, BVG)

In a correspondence analysis of all three sites, microbial communities grouped by site (McLaughlin sites lie to the upper left of the Bear Valley site) and by site type (goatgrass sites lie to the right of the starthistle site) (Figure 2). No overall separation by year or plant type (invasive versus native) was observed in Figure 2. A total of 47 fatty acids were used to generate this ordination, and 56.0% of the sample variation due to fatty acid content is explained in the first two axes. This CA was generated to explore the question: At what spatial scale are differences in invaded and native rhizosphere microbial communities observed? Two sub-sites were excluded from this analysis: MLG sub-site A (2001) and MLS sub-site E (2001) because they contained samples which differed greatly in fatty acid content compared to the rest of the samples (e.g. they were highly enriched in the fatty acid, i18: 0). Including these sub-sites in the analysis caused all of the samples shown in Figure 2 to group together in a single clump, obscuring the differences shown in Figure 2. Although MLG sub-site A (2001) and MLS sub-site E (2001) were not included in this CA, these sub-sites were analyzed on an individual basis to examine differences between invaded and native soil microbial community composition.

Canonical Correspondence Analysis (CCA), performed using site, site type (goatgrass versus starthistle), year, and plant type (invasive versus native) as categorical environmental variables revealed that each of these variables significantly

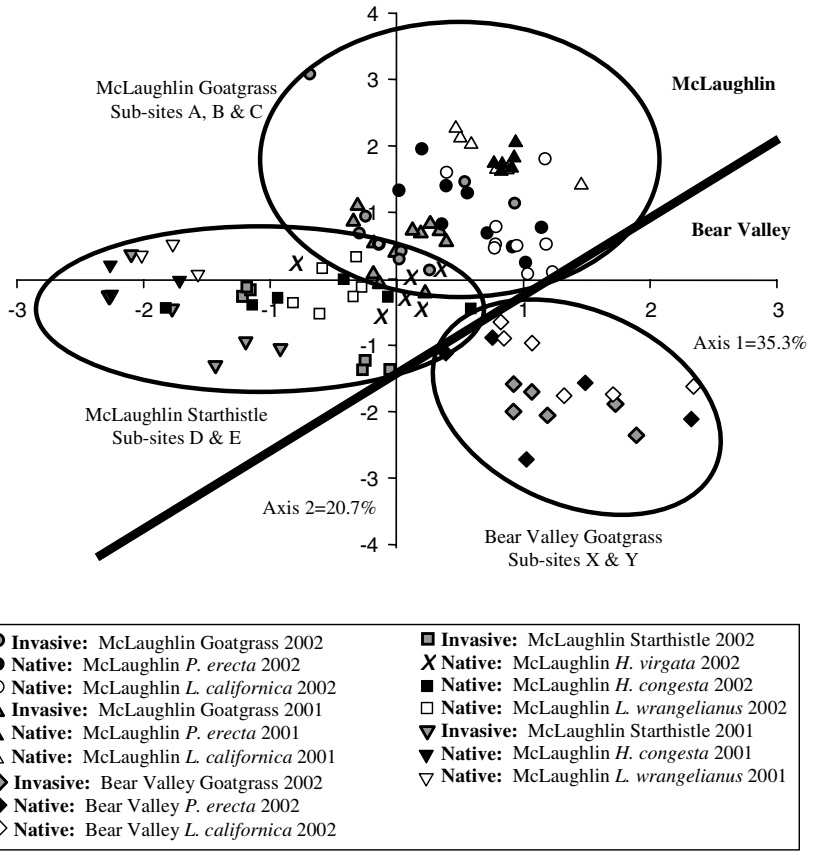

Figure 2. Correspondence Analysis (CA) of McLaughlin and Bear Valley Sites. The first two axes explain 56.0% of the sample variation due to phospholipid fatty acid content.

explained the ordination ($P \leq 0.05$, Monte Carlo Permutation Test). In the CCA, the samples grouped in the exact same fashion as depicted in Figure 2, and 89.4% of the sample variation due to fatty acid and environment data was explained in the first two axes (data not shown). Thus, not only the easily observed differences in sample grouping (site and site type) were significant in explaining the ordination, but year and plant type were also important explanatory variables. The large differences in fatty acid content in MLG sub-site A (2001) versus (2002) and MLS sub-site E (2001) versus (2002) further suggests that year is an important factor in determining microbial community composition.

Comparisons within sites

Significant changes in microbial community composition were evident after invasion by starthistle or goatgrass. In a comparison of microbial communities at the McLaughlin starthistle site (MLS sub-site D), the first two axes of the CA explain 76.6% of the variation with the same 47 fatty acids used in Figure 2 (Figure 3). Samples group by year along the *x*-axis and by plant type (invasive versus native) classifications along the *y*-axis. Based on a CCA of the same data using plant type as an explanatory variable, the invaded and native microbial communities significantly differ ($P \leq 0.05$, Monte Carlo permutation test, data not shown). There is greater separation of invaded and native samples in 2001 than in 2002. In 2001, three of the starthistle rhizosphere samples, taken at the edge (invasion front) of the starthistle patch, group with the native samples. Therefore, in 2001, the more newly invaded soil microbial community (at the edge of the patch) is more similar to the native soil microbial community than the soil microbial community that has been invaded for several years (in the center of the patch).

In a comparison of microbial communities at two goatgrass sites in the McLaughlin Reserve (MLG sub-sites B and C), the first two axes of the CA explain 53.9% of the sample variation due to fatty acid content (Figure 4). As in Figure 3, there is separation by year between the native plant rhizosphere samples; however, there is no yearly separation between the goatgrass rhizosphere samples. Also as in Figure 3, goatgrass center and edge rhizosphere samples separated, with the edge samples being slightly more similar in microbial community composition to the native plant samples than were the center patch samples. A CCA revealed that there is a signifi-

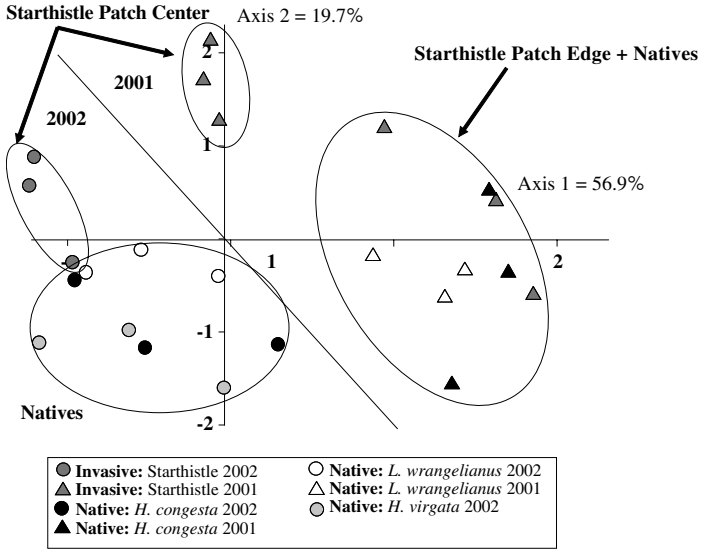

Figure 3. CA of McLaughlin Starthistle (MLS) Sub-site D, April 2001 and 2002. The first two axes explain 76.6% of the sample variation due to phospholipid fatty acid content.

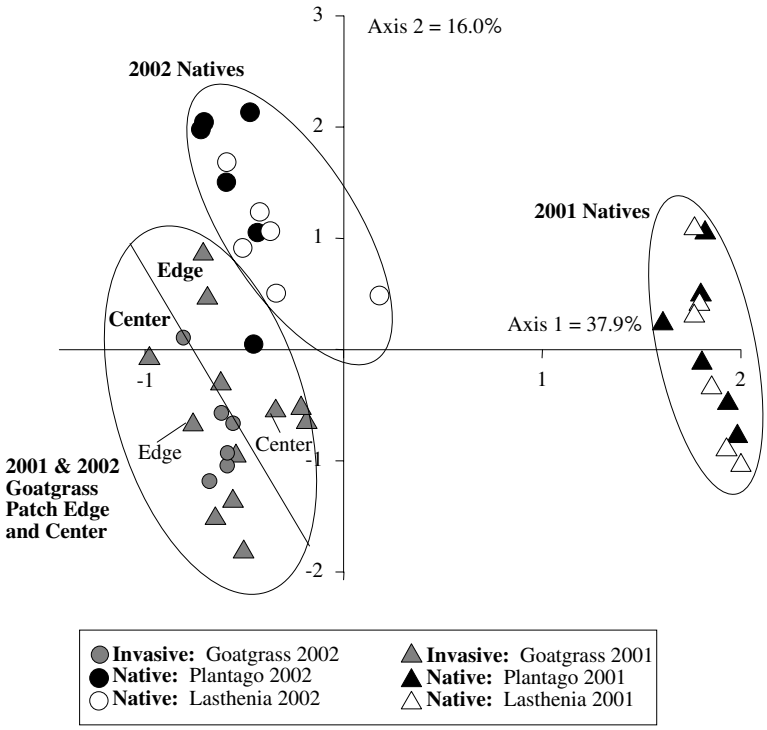

Figure 4. CA of McLaughlin Goatgrass (MLG) Sub-sites B & C, April 2001 & 2002. The first two axes explain 53.9% of the sample variation due to phospholipid fatty acid content.

cant plant type by year interaction and that the invaded and native microbial communities significantly differ ($P \leq 0.05$, Monte Carlo permutation test, data not shown).

Starthistle and goatgrass invasions appeared to change the local soil microbial community despite yearly variation in all soil microbial communities except those associated with goatgrass (Figures 3–4). All other individual sub-site CAs showed similar results with the exception of MLG sub-site A (2002) and MLS subsite E (2001) which showed no separation by plant type.

Comparison of individual fatty acid biomarkers

Biomarker fatty acids, total microbial biomass, and fatty acid diversity in invasive patch center ('center'), invasive patch edge ('edge'), and native ('native') samples were compared (Figures 5 and 6). Reflecting the transitional nature of the invasive plant patch edge (invasion front), edge samples sometimes were more similar to the center samples, sometimes intermediary between center and native samples, and sometimes different from either center or native samples. No differences in total microbial biomass between sample types were observed.

Starthistle center and edge samples contained higher amounts of 10Me16:0 (biomarker for sulfate reducing bacteria and actinomycetes) than the native samples (Figure 5). However, for all other biomarker and fatty acid diversity comparisons, edge samples were different from either of the other two sample types.

In comparisons within the MLG and BVG sites, goatgrass edge samples contained higher fatty acid diversity, and higher concentrations of biomarkers for AMF/Gram (–) bacteria/Type I methanotrophs (16: 1ω5c), and *Desulfovibrio* (sulfate reducing bacteria – i17:1) than native samples, while center samples had intermediate levels of these biomarkers (Figure 6). Edge samples contained intermediate levels of the biomarker for *Thiobacillus* (sulfur oxidizing bacteria – i17: 1ω5c).

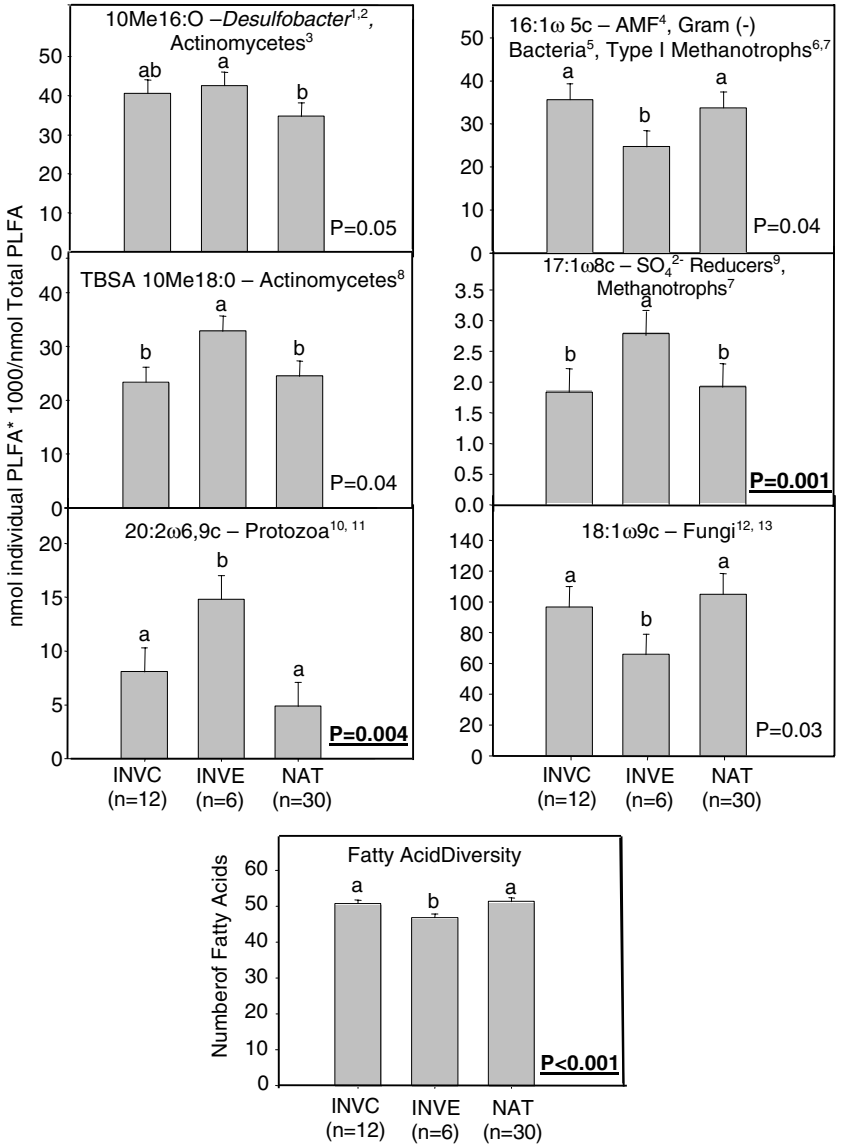

Figure 5. Significant biomarker fatty acid and fatty acid diversity comparisons within the starthistle site (MLS). INVC = starthistle center patch, INVE = starthistle patch edge, NAT = *L. wrangelianus*, *H. congesta*, *H. virgata*. Bars are depicted with standard errors. Bars sharing the same letter are not statistically different. Underlined values indicate probability less than sequential Bonferroni-corrected Type I error rate. 1 Dowling et al. (1986); 2 Dowling et al. (1988); 3 Al-Zarban et al. (2002); 4 Olsson et al. (1995); 5 White et al. (1996); 6 Nichols et al. (1985); 7 Holmes et al. (1999); 8 Linos et al. (1999); 9 Macalady et al. (2000); 10 White et al. (1996); 11 White et al. (1997); 12 Lindahl et al. (1997); 13 Schutter and Dick (2001).

Discussion

Differences in total invaded and native microbial community composition

Across all sampled locations, site (McLaughlin sites versus the Bear Valley site), site type (goatgrass versus starthistle sites), year, and plant type (invasive versus native) significantly influenced microbial community composition. Two subsites, MLG sub-site A (2001) and MLS sub-site E (2001) differed greatly in microbial community composition from the rest of the sub-sites, suggesting that year may be a more important

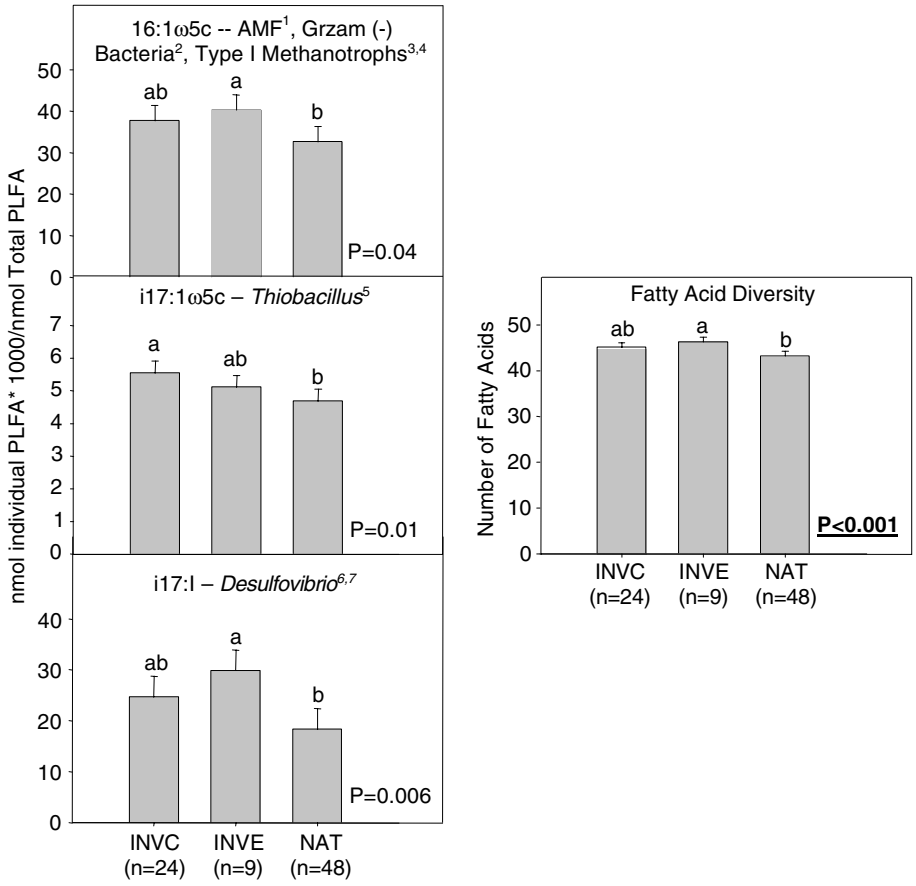

Figure 6. Significant biomarker fatty acid and fatty acid diversity comparisons in goatgrass (MLG and BVG) sites. INVC = goatgrass center patch, INVE = goatgrass patch edge, NAT = *L. californica*, *P. erecta*. Bars are depicted with standard errors. Bars sharing the same letter are not statistically different. Underlined values indicate probability less than sequential Bonferroni-corrected Type I error rate. 1 Olsson et al. (1995); 2 White et al. (1996); 3 Nichols et al. (1985); 4 Holmes et al. (1999); 5 Kerger et al. (1986); 6 Edlund et al. (1985); 7 Dowling et al. (1988).

explanatory variable for soil microbial community composition than plant type in some cases. Additionally, analyses of individual sub-sites revealed strong differences in microbial community composition by year. This yearly variation is likely due to the fact that the 2001–2002 growing season was much wetter throughout the year and warmer on average in the three months preceding sampling than was the 2000–2001 season (data not shown). Microbial community composition is known to respond to temperature and moisture (Bossio and Scow 1998; Grayston et al. 2001). Interestingly, goatgrass soil did not exhibit the same yearly variation observed in the other rhizosphere soils.

At an individual sub-site scale, plant type is an important determinant of microbial community composition, and both starthistle and goatgrass appear to be changing the soil microbial community composition in the areas they invade. Goatgrass and starthistle plant patches changed and/or expanded their ranges from 2000 to 2003; thus we conclude that these invasions are not confined to the patches due to pre-existing differences in soil chemistry and/or texture between invaded and native areas. In addition, a subsequent greenhouse study has shown that goatgrass changes the soil microbial community under controlled soil chemistry conditions (Batten 2004). This evidence leads us to believe that goatgrass

and starthistle are causing, rather than responding to, these changes in soil microbial community composition. Although the differences between invasive and native plant rhizosphere microbial communities may be small on a landscape scale, these differences may have an important cumulative effect on microbially-driven, ecosystem-scale processes such as nutrient cycling.

Plant invasions have been shown to alter soil carbon, nitrogen, salinity, moisture, and pH (Ehrenfeld 2003), all of which can impact soil microbial community composition. For example, the invasive tree, *Myrica faya*, has increased nitrogen fixation by 9000% in highly invaded areas in Hawaii (Vitousek and Walker 1989), and experimental N additions have been shown to change microbial community composition in Hawaiian soils (Balser 2001) and decrease microbial enzyme activities and biomass in a Michigan hardwood forest (Deforest et al. 2004). Soil aggregation and erosion processes impact microbial physical habitat and are also affected by plant invasion. For example, invasive annual grasses (including goatgrass) can either increase or decrease soil aggregate stability (Rillig et al. 2002; Eviner and Chapin 2002; Batten 2004).

Differences between native and invasive plant exudates, including allelopathic compounds, also likely impact root-associated microbial communities. Congeners of starthistle (*Centaurea maculosa*, *Centaurea diffusa*, and *Centaurea repens*) have all been shown to contain allelopathic compounds such as (±) catechin, sesquiterpene lactones (including cnicin), and polyacetylenes (Muir and Majak 1984; Stevens 1986; Kelsey and Locken 1987; Locken and Kelsey 1987; Bais et al. 2002). The allelopathic chemicals excreted by *C. maculosa*, a noxious weed in the western US, have been shown to contribute to its competitive dominance over *Festuca idahoensis*, a native bunchgrass (Ridenour and Callaway 2001). Additionally, an allelopathic compound exuded by *C. maculosa* is an enantiomer, (±) catechin; (−) catechin is a phytotoxin whereas (+) catechin is antibacterial (Bais et al. 2002). Thus *C. maculosa* may impact plant and microbial community composition through exudation of (±) catechin. Since it is related to these other *Centaurea* species, starthistle may also excrete allelopathic chemicals which may impact rhizosphere microbes and native plant fitness; no studies have yet addressed this question.

Invasive edge samples

In individual biomarker fatty acid comparisons, invasive edge samples can be more similar to invasive center samples, intermediate between native and longer-invaded samples, or frequently unlike either native or center samples. The unique biomarker composition of invasion front samples may reflect a short-lived perturbation in biomarker fatty acids in response to invasion; most biomarkers appear to return to similar levels once soils have been invaded for several years. It is important to note, however, that although individual biomarker fatty acids appear to exhibit this short-lived response, the *overall* microbial community composition in invasive edges is intermediate between those in invasive center patches and in native samples. Starthistle edge samples are more similar to native rhizosphere samples, whereas goatgrass edge samples are very similar to goatgrass center patch samples. Thus, goatgrass may alter the soil community more quickly than starthistle; this may have implications for the speed of restoration if these changes decrease native plant fitness and/or impact ecosystem function in invaded areas.

Potential changes in soil nutrient cycling and microbial functional groups with invasion

Changes in soil microbial community composition can be directly related to changes in nutrient cycling since soil microorganisms drive many of these cycles. However, differences in biomarker fatty acid concentrations must be interpreted with caution: different microbial species of the same genera can contain different amounts of specific biomarker fatty acids in their cell membranes and a single species can possess different percentages of a biomarker when grown on different carbon substrates (Dowling et al. 1986). Additionally, certain fatty acids can be biomarkers for more than one group of organisms; for example 10Me16:0 is found in both sulfate reducing bacteria and actinomycete membranes (Dowling et al. 1986, 1988; Al-Zarban et al. 2002).

Finally, biomarkers occasionally are not present in certain species of the genera they are supposed to represent. A compilation of pure culture fatty acid data for 100 isolates of sulfate reducing bacterial genera found that 10Me16:0 and i17:1 mostly were present in the genera of which they are indicative but were absent in a few isolates where they 'should' be and present in a few isolates where they 'should not' have been (Macalady et al. 2000). However, despite these caveats, biomarker fatty acids can be used to generate hypotheses about changes in nutrient cycling and ecosystem function which can then be tested by future studies.

Sulfur cycle

In goatgrass sites, concentrations of biomarkers for both sulfate-reducing and sulfur-oxidizing bacteria tended to be higher in edge and center patch samples than native plant rhizospheres and may represent changes in the sulfur cycle. It was once believed that sulfate-reducing bacteria (SRB) were obligate anaerobes, but recent studies have detected them in oxic and microaerophillic habitats and have shown that some of them can use oxygen as an electron acceptor (Minz et al. 1999; Ito et al. 2002). Thus, even in highly drained serpentine soils, SRB may act as facultative aerobes in oxic regions and may reduce sulfate in anoxic microsites.

Sulfur is an important plant nutrient; it is used in the production of the amino acids cysteine and methionine, proteins, coenzymes and secondary plant products and is typically taken up from the soil in the divalent anion form, SO_4^{2-} (Marschner 1995). An analysis of soil chemistry for MLG soils revealed significant differences in soil sulfate levels between goatgrass and native rhizosphere soil (mean goatgrass soil sulfate 3 ppm, ($n = 6$), mean native soil sulfate 11 ppm, ($n = 6$) ($P = 0.02$, one-way ANOVA, data not shown). A study of 17 non-serpentine California subclover-annual grass pastures found critical soil sulfate levels of around 5–8 ppm, above which plant yield leveled out with increasing soil sulfate concentrations (Vaughn et al. 1987). According to these determinations, sulfate may be limiting in MLG goatgrass-invaded soils but not limiting in MLG native soils.

Fatty acid diversity

Though the number of fatty acids in a sample does not directly correspond to the number of microbial species in that sample, its magnitude reflects microbial diversity. The number of fatty acids decreased significantly in the edge samples in the starthistle sub-sites; whereas the number of fatty acids was significantly higher in goatgrass edge samples. A study using substrate utilization (Biolog plates) as a measure of microbial diversity in Great Basin grasslands invaded by *Hologeton glomeratus* found that microbial diversity was highest in invaded areas, decreased in the ecotone (or invasion front), and further decreased in the native areas (Duda et al. 2003).

Arbuscular mycorrhizal fungi

The biomarker 16:1ω5c is associated with AMF (Olsson et al. 1995) and also with Type I methanotrophic bacteria (Nichols et al. 1985; Holmes et al. 1999) and Gram (−) bacteria (White et al. 1996). We confirmed that both goatgrass and starthistle are colonized by AMF in our sites (data not shown). The biomarker 16:1ω5c increases significantly in goatgrass edge samples compared to native samples, but decreases in starthistle edge samples compared to center and native samples. A study of New Jersey hardwood forests found a significant increase in 16:1ω5c concentration with the invasion of *Microstegium vimineum* (an endomycorrhizal grass) (Kourtev et al. 2003). Goatgrass and/or starthistle invasion may alter AMF densities; however, the PLFA method does not give information about how the AMF community composition may be changing with invasion.

Protozoa

Protozoa feed selectively on different species of bacteria and can change microbial community structure (Bonkowski et al. 2000). Protozoa can also increase plant growth by changing the bacterial community towards one containing a higher percentage of plant growth-promoting rhizobacteria, which, in turn, produce indolyl-3-acetic acid, a plant growth-promoting hormone

(Bonkowski and Brandt 2002). Within the starthistle sub-sites, a biomarker for protozoa (20:2ω6c,9c) was significantly higher in edge samples than in center and native samples. Thus, the protozoa biomarker was highest at the edges of starthistle patches, but returned to a similar concentration after several years of invasion. No significant differences in this biomarker were observed for the goatgrass sub-sites. Perhaps, in the case of starthistle invasion, an increase in the protozoa biomarker in edge samples reflects a change in soil community trophic dynamics which may result in increased starthistle plant growth along the invasion front.

Implications for native plant fitness and ecosystem function

Changes in soil microbial community composition caused by plant invasion may have direct or indirect impacts on native plant fitness. If this invaded microbial community contains organisms that are pathogenic to native plants or lacks beneficial organisms that are necessary for native plant establishment and survival, this whole process may act as a mechanism of increased invasion. This new invaded soil community may be inhospitable to re-establishment and growth of native plants, resulting in a negative interaction leading to increased invasion.

A changed, invaded soil microbial community could also manifest as differences in rates of nutrient cycling and therefore impact ecosystem function. Knowledge of how invaded microbial communities impact ecosystem function and native plant fitness could be extremely valuable in understanding and combating plant invasions as well as restoring native plant communities and is the focus of our current research.

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