Plant invasion of native grassland on serpentine soils has no major effects upon selected physical and biological properties

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Abstract

Plant invasions alter soil microbial community composition; this study examined whether invasion-induced changes in the soil microbial community were reflected in soil aggregation, an ecosystem property strongly influenced by microorganisms. Soil aggregation is regulated by many biological factors including roots, arbuscular mycorrhizal fungal hyphae, and microbially-derived carbon compounds. We measured root biomass, fungal-derived glomalin-related soil protein (GRSP), and aggregate mean weight diameter in serpentine soils dominated by an invasive plant (Aegilops triuncialis (goatgrass) or Centaurea solstitialis (yellow starthistle)), or by native plants (Lasthenia californica and Plantago erecta, or Hemizonia congesta). Root biomass tended to increase in invaded soils. GRSP concentrations were lower in goatgrass-dominated soils than native soils. In contrast, starthistle dominated soil contained a higher amount of one fraction of GRSP, easily extractable immunoreactive soil protein (EE-IRSP) and a lower amount of another GRSP fraction, easily extractible Bradford reactive soil protein (EE-BRSP). Soil aggregation increased with goatgrass invasion, but did not increase with starthistle invasion. In highly aggregated serpentine soils, small increases in soil aggregation accompanying plant invasion were not related to changes in GRSP and likely have limited ecological significance.

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1. Introduction

Invasive plants can change the composition of the soil microbial community (Belnap and Phillips, 2001; Klironomous, 2002; Kourtev et al., 2002, 2003; Kuske et al., 2002; Duda et al., 2003; Batten et al., in press). Less understood are the effects of invaded soil microbial communities on ecosystem function and how these changes in ecosystem function may affect future invasions. One ecosystem property that is strongly influenced by microorganisms is soil aggregation.

Well-aggregated soils are desirable for plant growth because they have greater arability, higher water holding capacity, higher carbon and nutrient levels, and greater stability against erosion (Tisdall and Oades, 1982; Carter, 2002). If plant invasion changes soil aggregation via changes in soil microbial properties or processes, this would change the ‘quality’ of the soil, which could lead to the undesirable outcome of increased plant invasion of these unique soils. Serpentine soils have a low calcium:magnesium ratio, low nitrogen content, high heavy metal content, low water holding capacity, and host many endemic species (Kruckeberg, 1984; Brooks, 1987; Huenneke et al., 1990). This unusual chemistry and water limitation contribute to the greater relative resistance of serpentine soils to plant invasion compared with other soil types. The purpose of this experiment was to determine if two invaders, Aegilops triuncialis (barb goatgrass) and Centaurea solstitialis (yellow starthistle), change aggregation of serpentine soils in our study system.
Soil aggregate stability depends on many abiotic and biotic factors (including interactions between clay particles, carbon input, microbial activity, and roots) at different spatial scales (Tisdall and Oades, 1982; Jastrow et al., 1998). Arbuscular mycorrhizal fungi (AMF) increase soil aggregation in two ways: their hyphae hold soil particles together, and they produce glomalin, a compound quantified in soils as glomalin-related soil protein (GRSP), that helps bind soil particles together. Several studies have shown the importance of AMF relative to other soil aggregating agents. A path analysis indicated that external hyphae were the most important determinant of macroaggregates (Jastrow et al., 1998), while another study found that roots and hyphae had equally important effects on soil aggregation (Thomas et al., 1993). Another paper found that GRSP concentrations (rather than hyphae), root length, and percent plant cover are important determinants of percent water stable aggregates (%WSA) in soil (Rillig et al., 2002a). Thus, changes in AMF population size or structure which affect hyphal length and/or GRSP concentrations likely will have a direct and measurable affect on soil aggregate stability.

The three native plants examined in this study, Lasthenia californica, Plantago erecta, and Hemizonia congesta, form mycorrhizal associations (Eviner and Chapin, 2002). Our previous research revealed that invasion of goatgrass in serpentine soils was accompanied by an increase in soil concentration of 16:1ω5c, a putative phospholipid fatty acid (PLFA) biomarker for AMF (Olsson et al., 1995; Batten et al., in press). In contrast, 16:1ω5c did not increase with invasion of starthistle, suggesting that goatgrass invasion may increase AMF densities at these sites while starthistle invasion does not. Colonization of goatgrass and starthistle by AMF at these sites has been confirmed using the Trypan blue staining method (K.M. Batten, unpublished data).

Since AMF play a large role in soil aggregation, we hypothesized that soil aggregation and GRSP would increase with goatgrass invasion and not with starthistle invasion. We measured root biomass, GRSP concentrations, and aggregate mean weight diameter in soils invaded by goatgrass and starthistle and compared them to soils dominated by native annual species.

2. Materials and methods

2.1. Site description and sampling

Field sampling was conducted at the University of California McLaughlin Natural Reserve, located in the Northern California Coast Range. More information about the study site and design can be found in Batten et al. (in press). Soils were sampled from two serpentine grassland sites: the McLaughlin goatgrass (MLG) site and the McLaughlin starthistle (MLS) site. These sites consist of goatgrass or starthistle patches surrounded by a primarily native plant community. The goatgrass patch and starthistle patch included in this study are actively expanding their ranges at approximate rates of 0.5 and 3 m per year, respectively (Batten et al., in press). Thus, we conclude that: (1) the distribution of the invasive patches is not confined to a certain range within these sites, (2) the invasions are actively progressing, (3) the edges of these patches can be considered invasion fronts, and (4) differences observed in native and invaded soils are caused by invasion.

Soil samples were taken from inside one goatgrass patch in the MLG site and one starthistle patch in the MLS site and compared to soils of the surrounding native plant community, dominated by L. californica and P. erecta at the MLG site and H. congesta at the MLS site. All plants included in this field study are annual forbs except for goatgrass (an annual grass), and all species reproduce by seed. The sampling design results in pseudoreplication but was selected due to site topography. The invasive plant patches at these sites exist on hillsides and in relatively flat areas. In order to minimize confounding factors due to topography (such as texture and moisture) between native and invasive plant-dominated soils, we selected invasive and native plant-dominated soils in flat areas, and only one such patch existed at each site. To maximize sampling of within-site soil heterogeneity, we took samples spaced 1–2 m from one another in both the invasive- and native-dominated soils.

In April 2003, 12 cores (5.0 cm diameter, 7.5 cm depth) were taken from each soil type for a total of 48 cores. Half of these samples were wet-sieved and analyzed for root biomass immediately. The other half of the samples were air-dried and analyzed for percent water stable aggregate distribution and GRSP. The dimension of these soil core samples were selected because (1) the same size cores were being used for a concurrent nitrogen cycling experiment, and (2) roots and microbial activity in these annual-dominated communities are concentrated in the surface soil (top 10 cm) (Jackson et al., 1988).

2.2. Water stable aggregate size distribution analyses

Water stable aggregate (WSA) size separation was performed using a wet sieving method adapted from Elliott (1986). Soil samples were initially passed through a 10 mm sieve; a series of three sieves were then used to obtain four aggregate size classes: (1) >2000 μm (large macroaggregates), (2) 250–2000 μm (small macroaggregates), 53–250 μm (microaggregates), and <53 μm (clay and silt). Samples between 50 and 80 g were placed on a 2 mm sieve and submerged in room temperature deionized water for five minutes before sieving. Sieving involved moving the sieve up and down approximately 3 cm through the water 50 times in 2 min. All water and soil material that passed through the sieve were transferred to the next size sieve, and the sieving process was repeated. The different size aggregate fractions were oven dried (50 °C) and weighed.
A correction for sand and rock weight contained in the aggregates was performed according to Six et al. (1998). Subsamples of the dried aggregate fractions were shaken for 18 h in 5% hexametaphosphate, poured over the correct sieve size, dried, and weighed. These sand and rock weights were then subtracted from the water stable aggregate weights. %WSA was calculated by dividing the mass of water stable aggregates within a certain size class by the mass of the total soil sample times 100. The two largest size classes (>2000 and 250–2000 µm) were combined into one macroaggregate size class and mean weight diameter (MWD) was calculated using the following equation:

\[
\text{MWD} = ( (>250 \mu\text{m}) \times 5.125 \\
+ (53 - 250 \mu\text{m}) \times 0.1515 \\
+ (< 53 \mu\text{m}) \times 0.0275)/100
\]

### 2.3. Glomalin-related soil protein

Four different glomalin-related soil protein (GRSP) fractions were measured: easily-extractable Bradford reactive soil protein (EE-BRSP; previously termed EGG), Bradford reactive soil protein (BRSP; formerly termed TG), easily extractable immunoreactive soil protein (EE-IRSP; formerly IREEG), and immunoreactive soil protein (IRSP; formerly IRTG) as previously described (Wright and Upadhyaya, 1998; Rillig et al., 2002a; Rillig, 2004). Briefly, EE-BRSP was extracted with 20 mM citrate, pH 7.0 at 121 °C for 30 min; BRSP was extracted with 50 mM citrate, pH 8.0 at 121 °C for sequential rounds of 60 min each. After centrifugation, the supernatant was analyzed (1) using a Bradford assay to obtain EE-BRSP and BRSP fractions, and (2) with ELISA using the monoclonal antibody MAb 32B11 to obtain EE-IRSP and IRSP fractions.

### 2.4. Statistics

Multivariate analysis of variance (MANOVA) was used to compare levels of MWD and GRSP in two pairs of soils, starthistle versus native soil and goatgrass versus native soil, using JMP software (2000 SAS Institute, Inc., Cary, NC). When necessary, data were transformed to meet assumptions of homogeneity of variance and normality of residuals. Correlation tables were generated to aid in the selection of variables for inclusion in the models. MWD was included in both models rather than the WSA data because MWD is calculated from, and therefore, correlated with, the measured WSA fractions. EE-IRSP was included in the goatgrass-native model because it is highly positively correlated with all other measured GRSP fractions. In the starthistle-native model, EE-IRSP and EE-BRSP are positively correlated with IRSP and BRSP, respectively, and were both included because they are negatively correlated with one another.

Both MANOVAs were significant (\(P=0.02\) for goatgrass-native comparisons, and \(P=0.01\) for starthistle-native comparisons), so ‘protected’ univariate analyses of variance (ANOVA) were performed on the individual response variables included in the models. These protected comparisons provided strong support for differences between native and invaded soil samples, and their \(P\) values are presented in bold in Figs. 2 and 3. Univariate ANOVAs were also performed on the other fractions of GRSP and WSA fractions not included in the original model to examine relationships among glomalin fractions and between MWD and WSA fractions. Because they were not included in the original MANOVA, comparisons of these variables provide weaker support for differences, and these \(P\) values are presented in regular text in Figs. 2 and 3. Root biomass was measured on a separate set of soil cores from all other measurements, and, therefore, could not be included in the MANOVAs. Thus, univariate ANOVAs were also performed on root biomass data. Linear regressions were performed to explore further relationships between measured variables.

### 3. Results

#### 3.1. Root biomass

Starthistle-invaded soil contained significantly higher root biomass than native soil at \(\alpha = 0.10\) (Fig. 1). The goatgrass-native comparison shared the same, although non-significant, trend; root biomass increased with invasion.

#### 3.2. Soil aggregation

All soils (native and invaded) were very highly aggregated, with over 85% of the WSA in the macroaggregate fraction (>250 µm) (Fig. 2). Goatgrass-invaded...
soil had significantly greater MWD and %WSA in the macroaggregate size fraction than native soil (Fig. 2). The different %WSA fractions sum to 100; thus, native soil showed a corresponding significantly greater proportion of clay and silt than goatgrass-invaded soil. In contrast, no differences in MWD or %WSA fractions were observed with starthistle invasion.

3.3. Relationships among aggregation, glomalin-related soil protein, and root biomass

Although goatgrass-invaded soils were more highly aggregated than native soils, they contained lower concentrations of EE-BRSP, EE-IRSP, and BRSP (Fig. 3). Starthistle-invaded soils contained more EE-IRSP, less EE-BRSP, and equivalent IRSP and BRSP compared to native soils (Fig. 3).

In within-site regressions of MWD, GRSP fractions, and root biomass, the only significant relationships were observed in the MLG site: MWD was negatively correlated with the BRSP and EE-IRSP fractions ($R^2 = 0.39$, $P = 0.03$, and $R^2 = 0.51$, $P = 0.009$, respectively, data not shown) and non-correlated with the remaining GRSP fractions and root biomass. In the MLS site, MWD was not correlated with any of the GRSP fractions or root biomass. Root biomass was not correlated with any of the GRSP fractions in either site.

4. Discussion

4.1. Soil aggregate stability

As predicted, soil aggregation increased with goatgrass invasion but not with starthistle invasion, corresponding with previous data showing an increase of the PLFA biomarker for AMF accompanying goatgrass invasion but not with starthistle invasion (Batten et al., in press). Goatgrass invasion increased soil MWD by 6% in an already very highly aggregated soil. This increase in aggregation is statistically significant; however, these soils
are initially so highly aggregated that the ecological significance of a 6% increase in terms of soil quality and plant growth is questionable.

Our soils fall within the high range of aggregate stability reported for grassland soils. Studies of grassland soils in the United States found %WSA in the macroaggregate size class to be 50% (Nebraska), 60% (Ohio), 70–80% (Georgia), 85% (Kentucky), 85.2–91.5% (Illinois), and 77–90% (California) (Jastrow et al., 1998; Franzluebbers et al., 2000; Six et al., 2000; Eviner and Chapin, 2002; Rillig et al., 2002a,b).

The high level of aggregation in our soils was surprising. Since serpentine soils characteristically have a low water-holding capacity and our soils are rocky, we expected them to be less well aggregated than non-serpentine grassland soils. However, in the above-mentioned studies of grassland soil aggregation, Eviner and Chapin (2002) is the only study of serpentine soils and found the highest level of soil aggregation (90%) of these studies.

Abiotic forces and underlying soil mineralogy may be responsible for high aggregate stability in serpentine soils. Some serpentine soils are lateritic, and lateritic soils contain high amounts of iron and aluminum oxides that aid in the cementation of stable aggregates (Brooks, 1987; Kemper and Koch, 1966; Six et al., 2000). Additionally, high levels of magnesium, characteristic of serpentine soils, have been shown to contribute to serpentine soil ‘stickiness’ during the wet season and impermeability during drought (Brooks, 1987). Nonetheless, changes in plant or microbial community composition appear to have a small, but measurable impact on soil aggregation in these highly aggregated soils, perhaps attributable to an increase in the AMF hyphal network.

4.2. Relationships among MWD, glomalin-related soil protein, and root biomass

The general lack of relationships observed among MWD, roots, and GRSP measurements was surprising and likely is due in part to the overall high level of aggregation and abiotic controls on aggregation in these serpentine soils. The measured levels of GRSP are in line with those found in California, Montana, and Texas grassland soils (Wright and Upadhyaya, 1998; Rillig et al., 2002a,b; Lutgen et al., 2003).

The increase in MWD accompanying goatgrass invasion was positively associated with previous measurements of 16:1ω5c (biomarker for AMF), but was negatively correlated with two out of four GRSP fractions and non-correlated with root biomass. Because of the large role of AMF in soil aggregation and the demonstrated positive linear relationships between GRSP and aggregate stability, we expected to see GRSP concentrations increase with goatgrass invasion. However, in highly aggregated soils (aggregate stability >80%), this positive linear relationship dissolves, and no relationship between GRSP and soil aggregate stability exists (Wright and Upadhyaya, 1998).

MWD was unaffected by starthistle invasion, however, EE-IRSP increased in starthistle-invaded soils. Recent work more confidently links the immunoreactive fractions of GRSP (EE-IRSP and IRSP) to AMF origin than the non-immunoreactive fractions (Rillig, 2004). Thus, the increase in EE-IRSP in starthistle-invaded soil may reflect greater AMF colonization with invasion. However, Steinberg and Rillig (2003) found that the EE-IRSP fraction increased over time during incubation of soils and appears to be mobilized during microbial processing of soil organic matter. The observed differences in EE-IRSP may be due to differences in microbial decomposition between invaded and native soils rather than differences in AMF colonization. The EE-IRSP fraction does not consist of the most recently produced GRSP, contrary to the hypothesis presented in Steinberg and Rillig (2003).

A study examining GRSP and soil aggregation in carbonate-rich soils in Spain found that the different fractions of GRSP were either negatively- or non-correlated with water stable aggregates (Rillig et al., 2003). This study concluded that GRSP was not the main aggregate binding agent in these soils and that high carbonate levels contributed to aggregate stabilization. Abiotic factors (such as iron and aluminum oxides and magnesium) likely play a large role in the aggregation of the serpentine soils in our study and may contribute to the observed negative or lack of correlation between biotic soil aggregating agents and %WSA.

4.3. Plant invasion and soil aggregation

Several studies have investigated the response of soil aggregation to plant invasion. Eviner and Chapin (2002) found that serpentine surface soil (0–0.15 m depth) grown with monocultures of an invasive grass (Bromus hordeaceus) tends to have lower aggregate stability than soil associated with monocultures of native plants (P. erecta, L. californica, H. congesta, and Lotus wrightii), with about 3–10% decrease in surface soil aggregate stability with invasion. In contrast, aggregate stability increased (about 3–17%) in soils grown with monocultures of three invasive grasses (Avena barbata, A. triuncialis, and Taeniatherum caput-medusae) compared to soils grown with monocultures of two native forbs (Amsinckia douglasiana and Trifolium microcephalum) (Rillig et al., 2002a). Lutgen and Rillig (2004) found that percent cover of the invasive forb Centaurea maculosa (spotted knapweed) was significantly negatively correlated (R2=0.53) with BRSP and non-correlated with %WSA. Since %WSA (1–2 mm size class) in these Montana soils was high (70–80%), the authors concluded that this negative influence of spotted knapweed on GRSP did not translate into changes in soil aggregation.

Different invasive plants appear to have different impacts on soil aggregation (in parallel to the differences observed among native plant species or crops). The above-mentioned examples reveal that plant invasion can decrease, increase,
or have no effect on soil aggregation. Our results suggest that goatgrass increases soil aggregation compared to soil dominated by native vegetation while starthistle does not. Although the increase in soil MWD in our study was small, plant invasion may have a larger impact on soil aggregation in less well-aggregated soils. More research is needed to explore how invasive plants alter soil aggregation and to examine the importance of invasion-altered soil aggregate stability relative to other invasion-induced changes in ecosystem function.

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