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SEASONAL VARIATIONS IN PLANT SPECIES EFFECTS ON SOIL N AND P DYNAMICS

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Abstract. It is well established that plant species influence ecosystem processes, but we have little ability to predict which vegetation changes will alter ecosystems, or how the effects of a given species might vary seasonally. We established monocultures of eight plant species in a California grassland in order to determine the plant traits that account for species impacts on nitrogen and phosphorus cycling. Plant species differed in their effects on net N mineralization and nitrification rates, and the patterns of species differences varied seasonally. Soil PO_4^- and microbial P were more strongly affected by slope position than by species.

Although most studies focus on litter chemistry as the main determinant of plant species effects on nutrient cycling, this study showed that plant species affected biogeochemical cycling through many traits, including direct traits (litter chemistry and biomass, live-tissue chemistry and biomass) and indirect traits (plant modification of soil bioavailable C and soil microclimate). In fact, species significantly altered N and P cycling even without litter inputs. It became particularly critical to consider the effects of these multiple traits in order to account for seasonal changes in plant species effects on ecosystems. For example, species effects on potential rates of net N mineralization were most strongly influenced by soil bioavailable C in the fall and by litter chemistry in the winter and spring. Under field conditions, species effects on soil microclimate influenced rates of mineralization and nitrification, with species effects on soil temperature being critical in the fall and species effects on soil moisture being important in the dry spring.

Overall, this study clearly demonstrated that in order to gain a mechanistic, predictive understanding of plant species effects on ecosystems, it is critical to look beyond plant litter chemistry and to incorporate the effects of multiple plant traits on ecosystems.

Key words: California annual grasslands; nitrogen cycling; phosphorus cycling; plant litter chemistry; plant–soil interactions; plant species effects; plant traits; soil nutrients.

INTRODUCTION

Plant species differ in their effects on many ecosystem processes, and the ecosystem effects of vegetation change are often predictable based on plant traits (reviewed in Eviner and Chapin 2003*a*). Plant litter chemistry is one of the best studied mechanisms by which plant species influence ecosystems (e.g., Melillo et al. 1982, Taylor et al. 1989, Stump and Binkley 1993, Scott and Binkley 1997), but plants may also alter ecosystems through other mechanisms such as root exudation (Marschner 1995), root turnover (Aerts et al. 1992, Hobbie 1995), and "indirect traits" such as plant effects on soil microclimate (Van Vuuren et al. 1992, Mack and D'Antonio 2003*a*, *b*). Many of these traits are distributed independently from one another among species (Eviner 2004), so it is not surprising that our ability to understand and predict plant species effects on ecosystem processes is greatly enhanced by considering the effects of multiple traits (Shock et al. 1983, Wedin and Tilman 1990, Cheng and Coleman 1991, Steltzer and Bowman 1998, Bottner et al. 1999, Eviner and Chapin 2003*a*, Mack and D'Antonio 2003*a*, *b*).

The main objective of this study was to assess the relative importance of various plant traits in determining seasonal patterns of plant species effects on N and P cycling. The California annual grassland is an ideal system in which to address this objective because it allows us to sample the overall effects of multiple plant traits at small spatial and temporal scales. Mature plant density is up to 50 000 plants per square meter (Heady 1958), ensuring that the samples collected represent the effects of all plant traits, as well as the effects of a large number of individual plants. Species effects can be studied in a relatively short-term experiment because nutrient cycling rates are rapid (allowing for detectable changes in a relatively short time), and because a 1–2 year time scale is realistic and pertinent in this annual grassland, where vegetation composition often changes rapidly in response to yearly changes in weather or management (Talbot et al. 1939). Finally, strong

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seasonal changes in climate, plant allocation (Heady et al. 1991), and in controls over productivity (George et al. 1988, Heady et al. 1991) allow us to explore the controls of plant species effects on biogeochemical cycling over a broad range of conditions, making studies from one set of plots generalizable to many other systems.

In this study, we planted monocultures of eight dominant species in California annual grasslands and then related plant species effects on N and P cycling to direct plant traits (litter chemistry and biomass, shoot chemistry and biomass, root biomass) and indirect traits (plant species effects on soil bioavailable C, temperature, and moisture). These indirect traits are mediated by direct plant traits. For example, species effects on soil temperature are mediated by litter and live-plant biomass, whereas soil bioavailable C is probably mediated by root exudation and turnover (Eviner 2004). The focal plant species of this study have been shown to differ significantly in many of these traits, with traits varying independently from one another across species (Eviner 2004). Because many of these traits simultaneously impact biogeochemical cycling, we used a variety of approaches to test our hypotheses concerning the role of these multiple traits.

Hypothesis 1: Multiple plant traits are responsible for plant species effects on N and P cycling.—By studying the biogeochemical effects of species with different combinations of traits (Eviner 2004), we were able to use multiple regressions to assess the suite of traits that most strongly impact biogeochemical cycling. For example, the species that we studied included plants with similar litter chemistry (e.g., *Trifolium* and *Lupinus*, or *Avena* and *Bromus*), but different effects on soil bioavailable C and soil temperature. Similarly, *Lupinus, Amsinckia,* and *Bromus* have similar effects on soil bioavailable C, but differ in litter chemistry and in their effects on soil temperature. We expected that multiple plant traits can best account for species effects on N and P cycling due to these differences in trait combinations across plant species.

Hypothesis 2: Species effects on N and P cycling change seasonally, as does the relative importance of different traits in determining species effects.—We expected that litter chemistry would have its strongest effect on biogeochemical cycling early in the growing season, when the first rains induce a flush of decomposition and leach labile compounds out of litter. Exudation was hypothesized to strongly influence N and P cycling in the fall and winter, when most plant growth and allocation is below ground (Savelle 1977, Jackson et al. 1988, Heady et al. 1991). Soil moisture was expected to be important later in the growing season as the system begins to dry, and soil temperature was expected to be a critical factor in the winter.

Hypothesis 3: Soil microclimate is a major driver of species effects on N cycling.—We measured rates of net N mineralization and nitrification in the laboratory under constant temperature and moisture conditions, and under field conditions. By eliminating species effects on soil microclimate in one set of incubations, we were able to gain insights into the effects of temperature and moisture on N cycling. We expected that litter chemistry would be an important predictor of potential rates, but its effects would be masked by species effects on soil temperature and moisture in field incubations.

Hypothesis 4: Non-litter traits are important mediators of species effects on N and P cycling.—We examined species effects on N cycling with and without litter inputs, expecting that species differ in their effects on biogeochemical cycling even without litter inputs, and that the patterns of species differences without litter inputs are different than species patterns with litter inputs.

Together, these approaches provide a mechanistic understanding of the ecosystem effects of plant species and how they change due to different suites of traits and over different environmental conditions.

Methods

Experimental site

This research took place at the University of California Hopland Research and Extension Center in the northern coastal mountains of Mendocino County, 160 km north of San Francisco, California, USA, 39°00′ N latitude, 123°04′ W longitude (see Eviner [2004] for complete details). This area of California experiences a mediterranean climate, with hot, dry summers and cool, wet winters. Mean annual precipitation is 960 mm, which occurs October through May, with 75% of that falling between November and February. Temperatures also vary seasonally, with a mean temperature of 7.5°C December through February, whereas summer temperatures (July through September) average 21°C, with a mean daily maximum temperature of 33°C. The growing season begins in the fall with the first germinating rains and continues until late spring, when the rains cease and most plants senesce. The plots were established at an elevation of 395 m on a west-facing slope of approximately 13°, on a Sutherlin soil (a medium-texture loam derived from hard sandstone and shale, classified as an ultic haploxeralf; Gowans [1958]). This site is dominated by annual vegetation, including *Avena barbata, Bromus hordeaceus, Aegilops triuncialis, Taeniatherum caput-medusae, Bromus diandrus, Erodium botrys, Lupinus bicolor,* and *Trifolium subterraneum.*

Experimental setup

In the summer of 1997, 1-m^2 monoculture plots were established of eight annual species common in northern California grasslands. These include: four species of grasses (barbed goatgrass [*Aegilops triuncialis* L.], wild oats [*Avena barbata* Link.], soft chess [*Bromus hordeaceus* L.], and medusahead [*Taeniatherum caput-medusae* L.]), two forbs (filaree [*Erodium botrys* (Cav.) Bertol.] and fiddleneck [*Amsinckia douglasiana* A. D.C.]), and

two legumes (dove lupine [*Lupinus bicolor* Lindey] and maiden clover [*Trifolium microcephalum* Pursch]). Nomenclature follows Hickman (1993). *Amsinckia*, *Trifolium,* and *Lupinus* are native California species, whereas the other species are exotics that are now dominant components of these grasslands. Eight replicate plots of each species, as well as eight replicates of an unvegetated treatment, were planted in a randomized-block design with a 0.5-m buffer between plots. Plots were blocked along slope position.

These plots were established by minimizing the seed bank on a 30 × 60 m area. The area was mowed, and litter was removed and autoclaved at 200°C for 4 hours in order to kill any seeds in the litter layer. This autoclaved litter was bulked, and an equal portion of litter was placed on each plot after seeds were planted. Germination of the soil seed bank was stimulated prior to autumn rains by irrigating with 6.35 cm of water. The resident seed bank was allowed to germinate, as it would in a typical fall germinating rain, and then was killed with glyphosate (Roundup Original Herbicide, Monsanto, St. Louis, Missouri, USA). This was repeated once more to ensure minimal quantities of unwanted seeds. Seeds of *Avena, Bromus, Aegilops, Taeniatherum,* and *Erodium* were collected from the field site, whereas seeds were purchased from California sources for *Trifolium, Lupinus* (S&S Seeds, Carpinteria, California, USA), and *Amsinckia* (Valley Seed Service, Fresno, California, USA). Average seed addition rates mirrored typical plant density in the early spring (Heady 1958). Seeding rates were adjusted for each species in order to achieve approximately equal biomass per unit area across species, based on greenhouse trials (V. T. Eviner, *unpublished data*). Seeds were raked into the soil, covered with autoclaved litter, and allowed to germinate naturally with the fall germinating rains. Species composition was maintained by weeding of unwanted species throughout the duration of the experiment.

A separate set of monoculture plots ("no-litter plots") had no vegetation cover for the first year of the experiment, and was planted in the second year (fall 1998). This allowed for a comparison of plant species effects on N and P cycling with and without litter inputs in the 1998–1999 growing season. Results in the no-litter plots might have been influenced not only by the absence of litter, but also by the plots being fallow for a year. However, the one year fallow was not likely to affect our ability to determine the traits responsible for species effects on biogeochemical cycling because all measured traits except for soil temperature (which is mediated by litter quantity) were similar in with-litter and no-litter plots (Eviner 2004). There were eight replicate plots of each species/litter treatment.

Sample collection/harvests

Most sampling focused on the plots established in the fall of 1997. Soil and plant samples were collected from these plots at the end of the first growing season in April 1998, and at three time points during the second growing season: late autumn (December 1998, three weeks after the first germinating rains), late winter (February 1999, just before plants began their aboveground growth spurt), and late spring (April 1999, when the soil was drier and plants were at peak biomass and beginning to set seed).

Plots were divided into 100 10 × 10 cm quadrats, and for each of these harvests, two random quadrats were selected for sampling, avoiding the quadrats on the plot perimeter, and any that had been previously sampled, covered by litter bags, or disturbed by gophers. In each quadrat, for each harvest, aboveground biomass was clipped from a 10 cm diameter ring, dried at 60°C for 48 hours, and weighed. Root biomass was determined in the late-February harvest, after most root growth had occurred (Jackson et al. 1988), by taking a 3.7 cm diameter × 15 cm deep soil core, separating roots by the floatation method, drying roots at 60°C for 48 h, and weighing. Soil characteristics were assessed from 3.7 cm diameter × 15 cm deep soil cores, which were harvested from the area beneath each vegetation ring. These two cores for each plot were bulked, mixed, and put on ice. Due to time constraints, the no-litter plots were measured for their effects on N and P cycling only in February, with three species: *Trifolium, Taeniatherum*, and *Avena* (eight replicates each treatment).

Litter quantity and quality

In July 1998, after all species had senesced, a 10 cm × 1 m strip of aboveground litter was harvested 10 cm from the left-hand edge of eight replicate plots, dried at 50°C for 48 h, and then weighed. Subsamples were ground in a Wiley mill, milled to a powder with a rolling mill, and analyzed for C and N on a Carlo Erba CHN autoanalyzer. (Carlo Erba Instruments, Milan, Italy).

Soil measurements

Soil microclimate.—Soil moisture content was determined gravimetrically and soil temperature was measured with a Barnant hand-held thermometer using a K-type thermocouple (Barnant, Barrington, Illinois, USA) placed at a depth of 5.3 cm. Daily minimum temperatures were measured before sunrise and daily maximum temperatures were measured in the mid-afternoon. Temperatures were measured in four replicate plots of each plant species in December and March (one week prior to the April harvest), because only four replicate plots could be sampled before large diurnal changes in soil temperature masked species effects. Only one plot of each treatment was measured in February.

Soil bioavailable C index.—The soil bioavailable C pool is a measure of microbial utilization of easily available soil C, and serves as an indicator of both the quantity and quality of soil C. These bioavailable C pools are independent of plant litter inputs and are probably an indicator of plant C inputs through root exudation and turnover (Eviner 2004). To perform this

assay, we picked roots and rocks out of a soil sample. We incubated 50 g (wet mass) of soil at 22°C at field moisture levels, in an airtight mason jar. CO_2 accumulation was determined at day 4 by analyzing air samples for CO_2 on a Shimadzu gas chromatograph (GC 11A), using a thermal conductivity detector (Shimadzu Scientific Instruments, Columbia, Maryland, USA).

Net N mineralization and nitrification.—Rates of net N mineralization and nitrification were determined in two separate incubations at each sampling time. Potential rates were determined under constant temperature and moisture conditions in the laboratory, and served as an indicator of plant effects on the quality of organic matter and its potential as a resource pool for the soil microbial community. A second subsample of soil was simultaneously incubated in the field, reflecting species effects on N cycling due to plant effects on soil organic matter, temperature, and moisture. Both incubations lasted for one week, and thus reflected the impact of the most active form of soil organic matter (Bundy and Meisinger 1994). Other studies have shown that differences among plant species dissipate quickly in lab incubations compared to natural conditions where the plants remain present (Wedin and Pastor 1993), confirming the importance of a short-term assay.

Immediately after soil samples were collected, soils were hand-mixed, and ~100 g of soil were placed into a polyethylene bag and placed back into the hole from which the soil had been taken. The remaining soil sample was put on ice and brought back to the lab. A subsample was immediately extracted with 0.5 mol/L K_2SO_4 to determine initial concentrations for both field and lab samples, and to determine in situ inorganic N pools. A second subsample of soil was brought to constant moisture and allowed to incubate in the lab for one week. In December and February, soils were incubated at 22% moisture, which is just below field capacity for these soils. Soils that were too wet were allowed to air dry until they reached the desired mass (usually within 2–6 hours). In April, the soils were much drier and were only wet to 14% moisture (the highest soil moisture content of any of the samples) in order to better reflect the dry spring conditions and to minimize the disruption of microbial membranes that could release a flush of C and N (Bottner 1985). As a result of these different moisture levels, the April measurements of potential net rates cannot be directly compared to those from the December and February time points. All lab samples were incubated at room temperature (22°C). After a 7-d incubation, both lab and field soils were extracted with 0.5 mol/L K_2SO_4. All soil-K_2SO_4 mixtures were shaken on a mechanical shaker for one hour, and then allowed to sit in the cold room (4°C) overnight. These extracts were then filtered using preleached Whatman No. 1 filter paper. The extracted soil was then wet-sieved to determine rock and gravel content >2 mm diameter. Extracts were analyzed for concentrations of NH_4^+ (Lachat QuikChem Method 12–107–06–2-A) and NO_3^- (Lachat QuikChem Method 12–107–04–1-B) on a Lachat autoanalyzer (flow-injection analyzer QC 8000, Lachat Instruments, Milwaukee, Wisconsin, USA). Inorganic nitrogen concentrations per gram of dry soil were determined by correcting the extracted soil mass for moisture and rock content. Net rates of N mineralization and nitrification were determined by subtracting initial concentrations of NH_4^+ and NO_3^- from post-incubation concentrations.

Soil PO_4^- and microbial P.—Soil PO_4^- concentrations were determined at each time point by extracting 5 g of soil (wet mass) with 35 mL of Bray #1 solution, which has been shown to be the best indicator of plant available P at this field site (Vaughn and Jones 1980). Extracts were shaken by hand for 60 s and were filtered through Whatman #42 filter paper. Soil PO_4^- concentrations were determined colorimetrically by phosphomolybdenum-blue color formation and manual analysis on a spectrophotometer (Beckman DU-2, Beckman Coulter, Fullerton, California, USA). Microbial biomass P was determined by chloroform fumigation extraction. Soils were fumigated with ethanol-free chloroform for 24 hours. A comparison of fumigated samples that were only extracted with those that had been digested with $HClO_4$ and HNO_3 indicated that 80% of the P released from the microbial biomass was released as PO_4^-. This percentage did not differ among plots dominated by different plant species (C. E. Vaughn, unpublished data), so fumigated samples were simply extracted and not digested.

Statistical analyses

All statistics were determined using JMP 4.04 software (SAS Institute 1989–2000). The effects of species, block, and interactions of species with block were determined by multiple analysis of variance (MANOVA), using plant species as the independent variable, and plot measurements of soil N and P cycling as the dependent variables. The effect of experimental block was significant only for the phosphorus data, with P increasing down the slope (V. T. Eviner and C. E. Vaughn, *unpublished data*). In order to remove the block effect, a regression line was fit with a cubic function between blocks and P concentration. This was possible because P varied by block (slope position) in a continuous manner. ANOVAs were then performed on the residuals to determine plant species effects.

The effects of species, season, and species × season interactions were determined by repeated-measures ANOVA. Unvegetated treatments were considered as a "species" treatment in these analyses. ANOVA, followed by a Tukey-Kramer post hoc test, was used to compare plant species effects at each time point for each process or to determine the effects of with-litter vs. no-litter plots for each species. This was an appropriate analysis in this data set because variances were usually equal, and ANOVA is robust even with slight departure

TABLE 1. Direct and indirect plant traits used in stepwise regressions to account for species effects on N and P cycling.

Variable	Net N mineralization		Net nitrification			
	Potential	Field	Potential †	Field ‡	Soil PO_4^-	Soil microbial P
Aboveground litter						
C:N	x	x	x	x	x	x
N (%)	x	x	x	x	x	x
C (%)	x	x	x	x	x	x
Biomass	x	x	x	x	x	x
Soil						
Bioavailable C	x	x	x	x	x	x
Aboveground live plant						
C:N	x	x	x	x	x	x
N (%)	x	x	x	x	x	x
C (%)	x	x	x	x	x	x
Biomass	x	x	x	x	x	x
Root biomass §	x	x	x	x	x	x
Root:shoot §	x	x	x	x	x	x
Total plant biomass §	x	x	x	x	x	x
Soil temperature						
Minimum		x		x	x	x
Maximum		x		x	x	x
Daily change		x		x	x	x
Soil moisture		x		x	x	x
Soil microbial P					x	
Soil PO_4^-						x

† Separate analysis included soil NH_4 and potential net mineralization.
‡ Separate analysis included soil NH_4 and field net mineralization.
§ Sampled in February only.

from this assumption when the experimental design is balanced (Zar 1996).

In order to determine the relationship between ecosystem processes and plant traits that influenced these processes (Table 1), we used stepwise multiple regression, making sure that the residuals were normally distributed. Regressions were performed using each species' mean values of traits and ecosystem processes, which is the standard way to examine the mechanisms determining plant species effects on ecosystems (McClaugherty et al. 1985, Aber et al. 1990, Scott and Binkley 1997). The regression analysis did not include the unvegetated plots because plant traits were absent there.

RESULTS

Seasonal effects of species on net N mineralization and nitrification

Plant species differed significantly in their effects on net N mineralization (Fig. 1). Species effects on field rates of net mineralization varied through the growing season (Fig. 1A, MANOVA; for species, $F_{8,63} = 4$, $P = 0.075$; for season, $F_{2,62} = 0.9$, $P = 0.41$; for species × season, $F_{16,\,124} = 3$, $P = 0.0005$). In particular, plant species differed in their effects on field net mineralization in December ($F_{8,71} = 4.3$, $P = 0.0003$) and February ($F_{8,71} = 2.7$, $P = 0.01$), but not in April ($F_{8,71} = 1.5$, $P = 0.19$), (Fig. 1A). The variations in species effects across the growing season were probably due to the fact that different traits were responsible for plant species effects at different times (Table 2). Species effects on field rates of N mineralization were most closely associated with soil temperature and moisture in December, bioavailable C and plant biomass in February, and litter C:N and soil moisture in April (Table 2).

Potential rates of net mineralization were assessed in the laboratory, under constant temperature and moisture conditions. Species and season had significant, independent effects on potential rates of N mineralization, and although some species effects did change with season, these were not statistically significant (Fig. 1B, MANOVA; for species, $F_{8,63} = 4.5$, $P = 0.0003$; for season, $F_{2,62} = 9$, $P = 0.002$; for species × season, $F_{16,\,124} = 1.5$, $P = 0.11$). Species differed significantly in their effects on potential rates of N mineralization in December (Fig. 1B, ANOVA; $F_{8,71} = 3.8$, $P = 0.001$), tended to differ in April (ANOVA; $F_{8,71} = 1.8$, $P = 0.08$), but did not differ in February (ANOVA; $F_{8,71} = 1.4$, $P = 0.22$). Species effects on potential rates of net N mineralization were correlated with bioavailable C in December, litter C:N in February, and litter percentage of N in April (Table 2).

Plant species effects on rates of net nitrification were stronger and more frequent than their effects on net N mineralization (Fig. 2). Field measures of net nitrification indicated that plant species effects significantly changed over the growing season, and that plant species and season also had significant independent effects on nitrification (MANOVA; for species, $F_{8,62} = 4$, $P =$

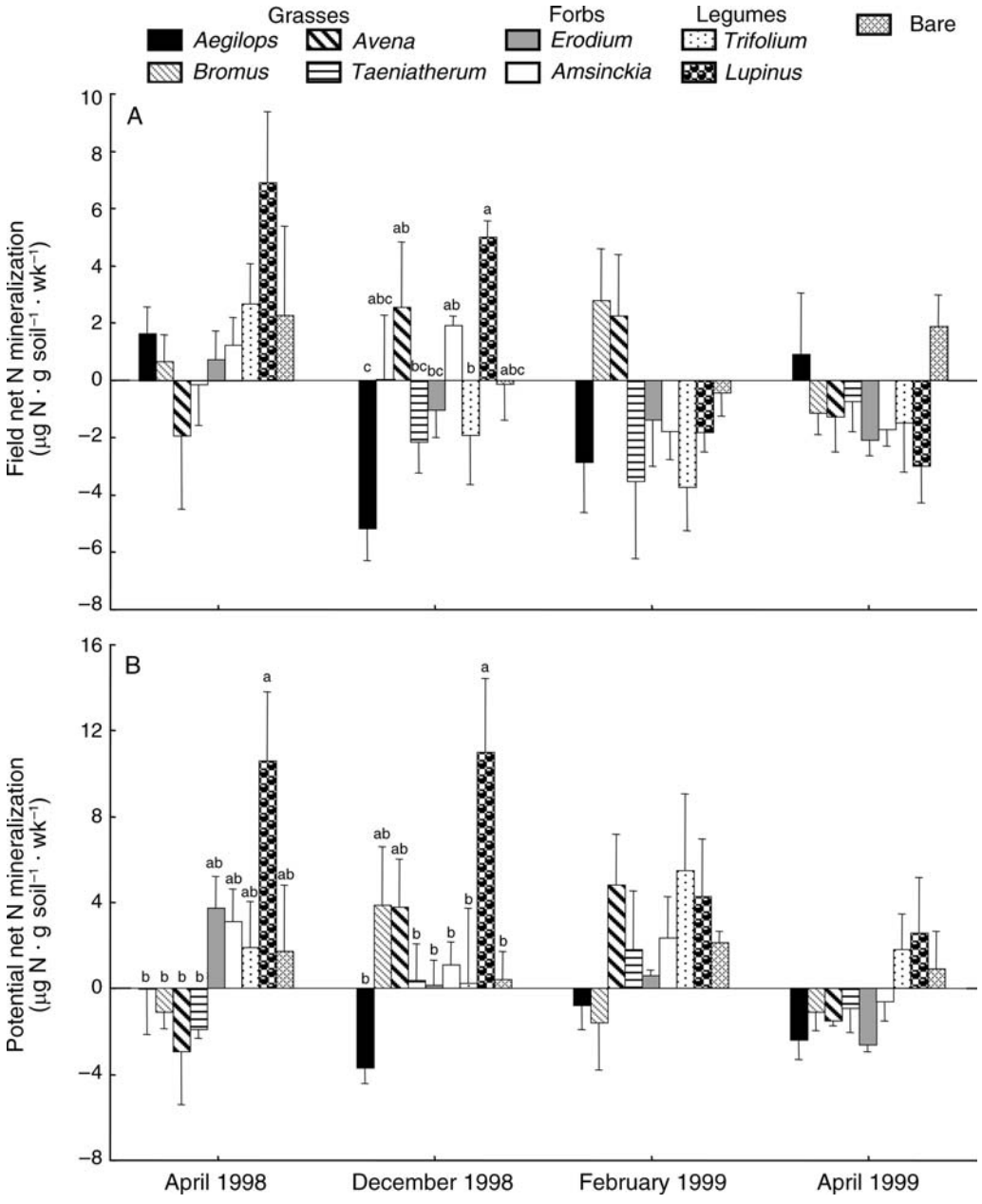

FIG. 1. Seasonal patterns of plant species effects on (A) field and (B) potential rates of net N mineralization per gram dry mass of soil. Data are means + SE ($n = 8$ replicate plots for each species and bare substrate treatment). Significant differences among species for each time point are designated by different lowercase letters (determined by a Tukey-Kramer post hoc test, $P < 0.05$). Although ANOVA indicates that species differed in their effects on N mineralization in February, the Tukey-Kramer test could not discern which species differed from one another at the $P = 0.05$ or $P = 0.10$ levels). Species are ordered from high initial litter C:N to low litter C:N.

0.0007; for season, $F_{2,61} = 22.5$. $P < 0.0001$; for species × season, $F_{16,\ 122} = 3.9$, $P < 0.0001$). Potential rates of net nitrification were significantly affected by plant species, and these species effects changed over the growing season, but season had only a weak independent effect on potential net nitrification (Fig. 2B, MANOVA; for species, $F_{8,62} = 11$, $P < 0.0001$; for season, $F_{2,61} = 2.3$, $P = 0.09$; for species × season, $F_{16,\ 122} = 1.7$, $P = 0.04$).

In the cold, wet part of the growing season (December and February), nitrification rates were lower in the field than in the lab, but the patterns of plant species effects on net nitrification were similar in the field and laboratory (Fig. 2). The relative rankings of species effects on net nitrification measured in the laboratory vs. the field only differed in April 1999. This contrasts with patterns of species effects on net mineralization, which

TABLE 2. Stepwise multiple regressions relating rates of net N mineralization and nitrification with plant traits (Table 1).

Response variable and explanatory variables	r^2	ss of factors	F	df	P	Relationship
December 1998						
Mineralization						
Potential	0.87		17.1	1,7	0.006	
Bioavailable C						+
Field	0.94		21.4	3,7	0.006	
Min. soil temperature		43.0	41.1		0.003	−
Soil moisture		25.3	24.3		0.008	+
Litter C (%)		9.7	9.3		0.04	−
Nitrification						
Potential	0.74		17.4	1,7	0.006	
Litter N (%)						+
Field	0.88		17.7	2,7	0.005	
Litter N (%)		12.5	34.9		0.002	+
Live biomass		2.7	7.5		0.04	−
February 1999						
Mineralization						
Potential	0.62		9.7	1,7	0.021	
Litter C:N						−
Field	0.92		28.4	2,7	0.002	
Bioavailable C		35	50.2		0.0009	−
Live biomass		24	34.0		0.002	+
April 1999						
Mineralization						
Potential	0.93		76.1	1,7	0.0001	
Litter N (%)						+
Field	0.87		8.6	3,7	0.03	
Litter C:N × soil moisture		2.4	7.9		0.05	
Litter C:N		2.0	6.7		0.06	−
Soil moisture		1.9	6.3		0.07	+
Nitrification						
Potential	0.84		32.0	1,7	0.0013	
Litter N (%)						+

Notes: Rows for potential and field mineralization and nitrification show statistics, including r^2, for the overall model; rows for the significant factors include ss (when there is >1 significant factor) and the direction of the relationship. Results for potential nitrification in February, field nitrification in February and April, and for all four measures in April 1998 are not shown because they are not significant.

were fundamentally different between lab and field incubations throughout the growing season. Litter percentage of N was the main trait accounting for species effects on both field and potential rates of net nitrification in December, and for potential rates in April. None of the measured traits significantly accounted for species effects on field and lab nitrification in February, or field nitrification in April (Table 2). When potential rates of net N mineralization were included as variables in stepwise regressions, they became the main explanatory variables of potential rates of net nitrification. However, field rates of nitrification only related to rates of net mineralization in the spring, not in the fall or winter.

Together, the laboratory and field measures of N cycling clearly demonstrated that traits beyond litter chemistry determine plant species effects on N mineralization and nitrification. In particular, species effects on soil microclimate are important drivers of species effects on net mineralization rates, by overwhelming the effects of plant C inputs (as in December), altering the effects of plant C inputs (as in April), or altering which plant C inputs have a dominant effects (as in February).

The importance of non-litter traits was particularly evident in the large differences in plant species effects in the first season, without any litter feedback, April 1998 (Figs. 1 and 2, ANOVA: for potential net mineralization, $F_{8,71} = 3.7$, $P = 0.001$; field net mineralization, $F_{8,71} = 1$, $P = 0.44$; potential net nitrification $F_{8,71} = 8.9$, $P < 0.0001$; field net nitrification $F_{8,71} = 5.4$, $P < 0.0001$). Patterns seen in the lab vs. the field were similar in April 1998, probably due to El Niño, which created a rare condition in California grasslands, where soil moisture and temperature were simultaneously ideal for plant growth and nutrient cycling. Species effects on rates of net N mineralization and nitrification prior to litter input were not significantly correlated with any plant traits measured (April 1998; Table 2).

The importance of traits other than litter chemistry was also evident by comparing with-litter and no-litter plots (Figs. 3 and 4). For measures of net mineralization in the field, neither with-litter or no-litter treatments showed significant differences among species, but litter treatments tended to alter rates of field net mineralization within a species treatment, with the direction of change differing among species (Fig. 3A, MANOVA;

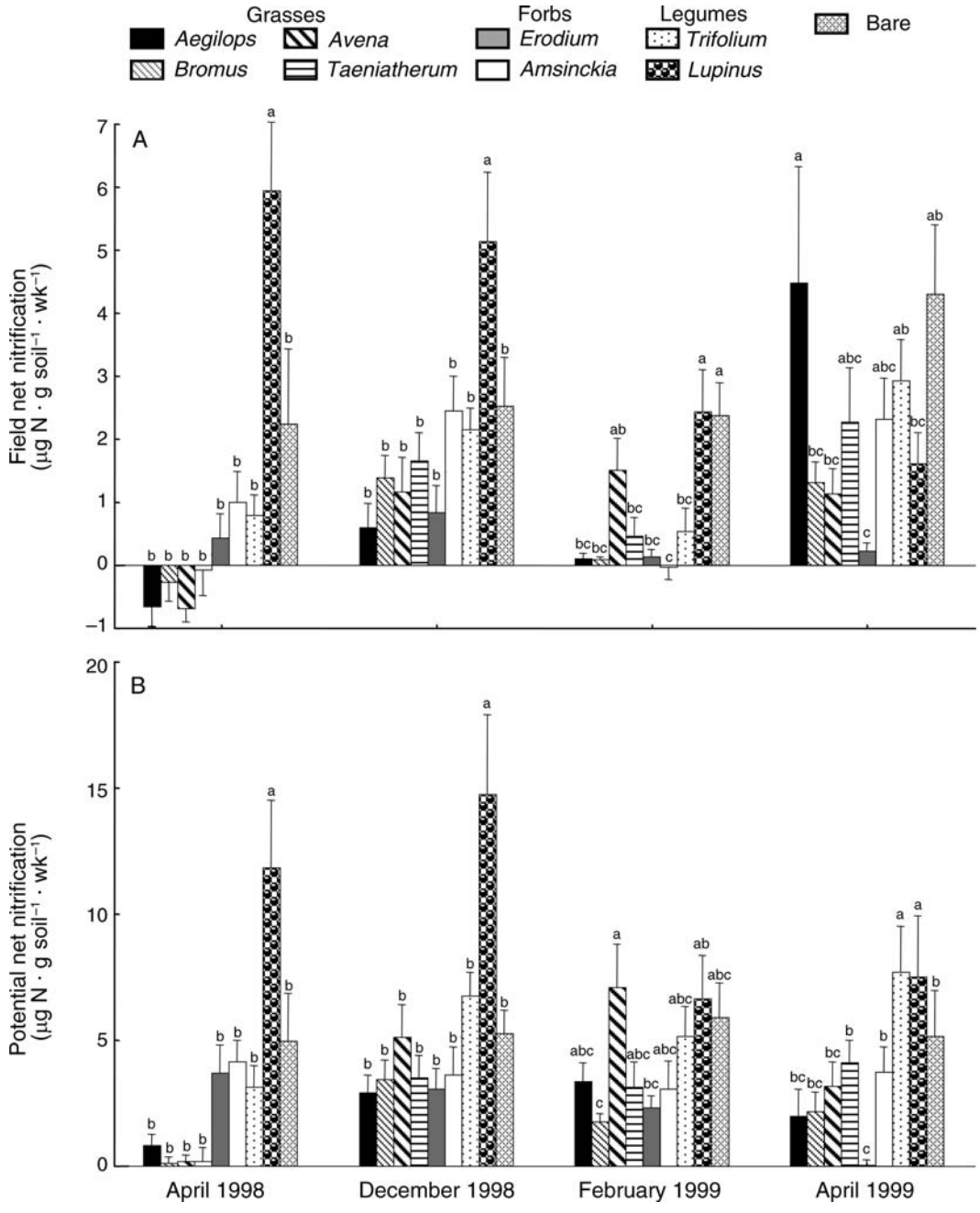

FIG. 2. Seasonal patterns of plant species effects on (A) field and (B) potential rates of net nitrification per gram dry mass of soil. Data are means + SE ($n = 8$ replicate plots for each species and bare substrate treatment). Significant differences among species for each time point are designated by different letters (determined by a Tukey-Kramer post hoc test, $P < 0.05$). Species are ordered from high initial litter C:N to low litter C:N.

for species, $F_{2,47} = 0.7$, $P = 0.51$; for litter, $F_{1,47} = 0.34$, $P = 0.92$; for species × litter, $F_{2,47} = 2.7$, $P = 0.08$). *Avena* tended to have lower rates of field net mineralization in the absence of litter (t test, $P = 0.08$), whereas the absence of litter tended to increase rates of net mineralization in *Trifolium* (t test, $P = 0.08$; Fig. 3A). Rates of potential net mineralization were lower in no-litter plots (Fig. 3B, MANOVA; for species, $F_{2,47} = 1.8$, $P = 0.18$; for litter, $F_{1,47} = 11$, $P = 0.0002$; for species × litter, $F_{2,47} = 0.32$, $P = 0.72$).

Species tended to differ in their effects on potential net nitrification in no-litter plots (Fig. 4B; $F_{2,23} = 3$, $P = 0.07$), but these patterns of species effects were quite different than those seen in with-litter plots (MANOVA;

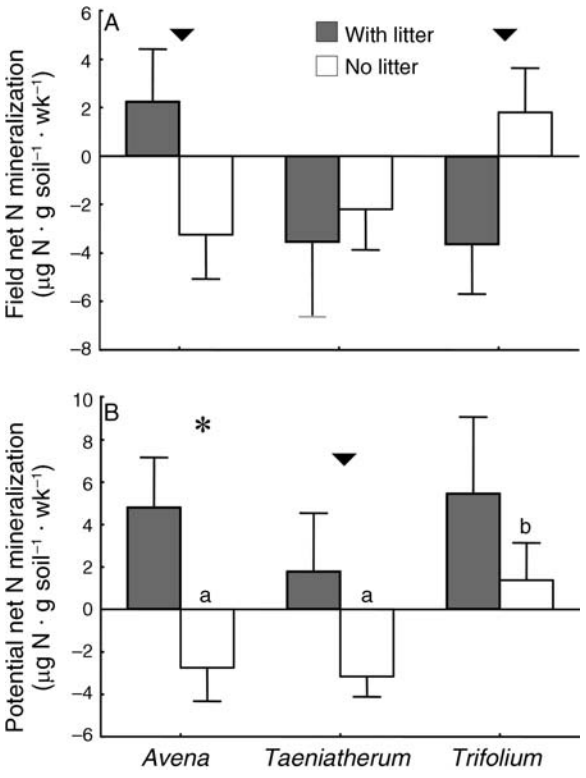

FIG. 3. Plant species effects on (A) field and (B) potential rates of net N mineralization per gram dry mass of soil in plots with (solid bars) and without (open bars) litter inputs in February. Data are means + SE ($n = 8$ replicate plots for each treatment). Significant differences among species for no-litter treatments are designated by different letters (determined by a Tukey-Kramer post hoc test, $P \leq 0.05$). Significant differences between with-litter and no-litter treatments within a species are designated by asterisks ($^*P \leq 0.05$); triangles indicate $P < 0.10$ (determined by t tests).

for species, $F_{2,42} = 6.6$, $P = 0.004$; for litter, $F_{1,42} = 193$, $P < 0.0001$; for species × litter, $F_{2,42} = 4.8$, $P = 0.0$). In field incubations, there also tended to be different patterns of species effects on net nitrification in the presence and absence of litter (Fig. 4A, MANOVA; for species, $F_{2,47} = 1.9$, $P = 0.17$; for litter, $F_{1,47} = 6.2$, $P = 0.02$; for species × litter, $F_{2,47} = 2.4$, $P = 0.10$).

Together, these results demonstrate that species effects on net N mineralization and nitrification were due to multiple plant traits. Litter chemistry and bioavailable C were related to potential rates of N cycling, and patterns of N cycling in the field correlated with these same C inputs, along with plant biomass and plant effects on soil microclimate. The importance of these non-litter traits is highlighted by significant species effects on N cycling even in the absence of litter inputs.

Phosphorus

The location of plots along the hill slope had a greater effect on soil phosphate concentration than did species (MANOVA; for species, $F_{8,63} = 0.8$, $P = 0.6$; for block, $F_{1,63} = 15.6$, $P = 0.0002$; for block × species, $F_{8,62} = 0.9$, $P = 0.53$), with PO_4^- increasing with downslope position. Phosphate concentrations varied with season, and species effects tended to change across seasons (Fig. 5A, MANOVA; for species, $F_{8,63} = 0.9$, $P = 0.6$; for season, $F_{2,61} = 24$, $P < 0.0001$; for species × season, $F_{16,122} = 1.5$, $P = 0.08$). Significant species differences were seen in February (ANOVA; $F_{8,72} = 2.2$, $P = 0.04$), with species effects on soil phosphate concentrations being positively correlated with litter C:N (Table 3).

Microbial P was not related to plant species or block (MANOVA; for species, $F_{8,62} = 0.14$, $P = 0.99$; for block, $F_{1,62} = 0.14$, $P = 0.99$; for species × block, $F_{8,62} = 1.3$, $P = 0.23$), but tended to change with season (Fig. 5B, MANOVA; for species, $F_{8,62} = 0.14$, $P = 0.99$; for season, $F_{2,62} = 18$, $P < 0.0001$; for species × season, $F_{8,62} = 1.9$, $P = 0.13$). Species differed significantly in their effects on microbial P in December (ANOVA; $F_{8,66} = 2.2$, $P = 0.04$), with these patterns being related to aboveground biomass and litter C:N (Table 3). Even without litter inputs, plant species significantly influenced microbial P (April 1998, ANOVA; $F_{8,71} = 2.2$, $P = 0.04$), with high microbial P found in soils associated with the grasses and *Amsinckia*, and low microbial P in *Erodium* and *Trifolium* (Fig. 5B).

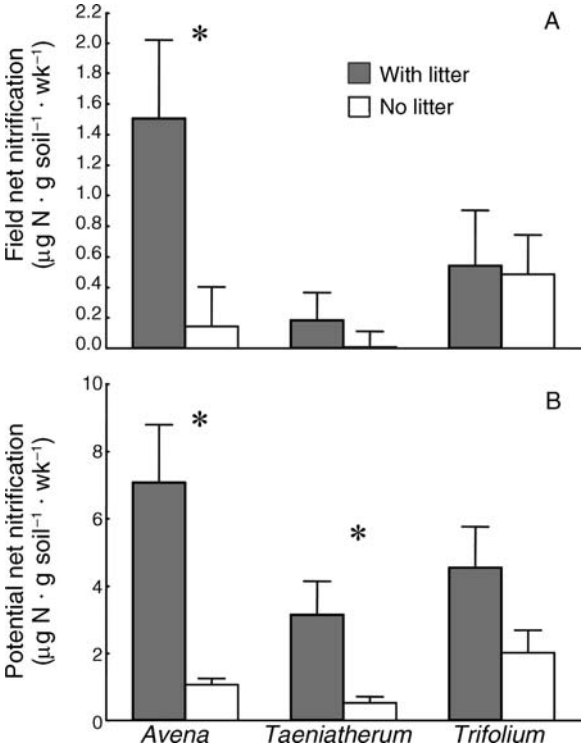

FIG. 4. Plant species effects on (A) field and (B) potential rates of net nitrification per gram dry mass of soil in plots with (solid bars) and without (open bars) litter inputs in February. Data are means + SE ($n = 8$ replicates per treatment). Significant differences between with-litter and no-litter treatments within a species are designated by asterisks ($^*P \leq 0.05$, determined by t tests).

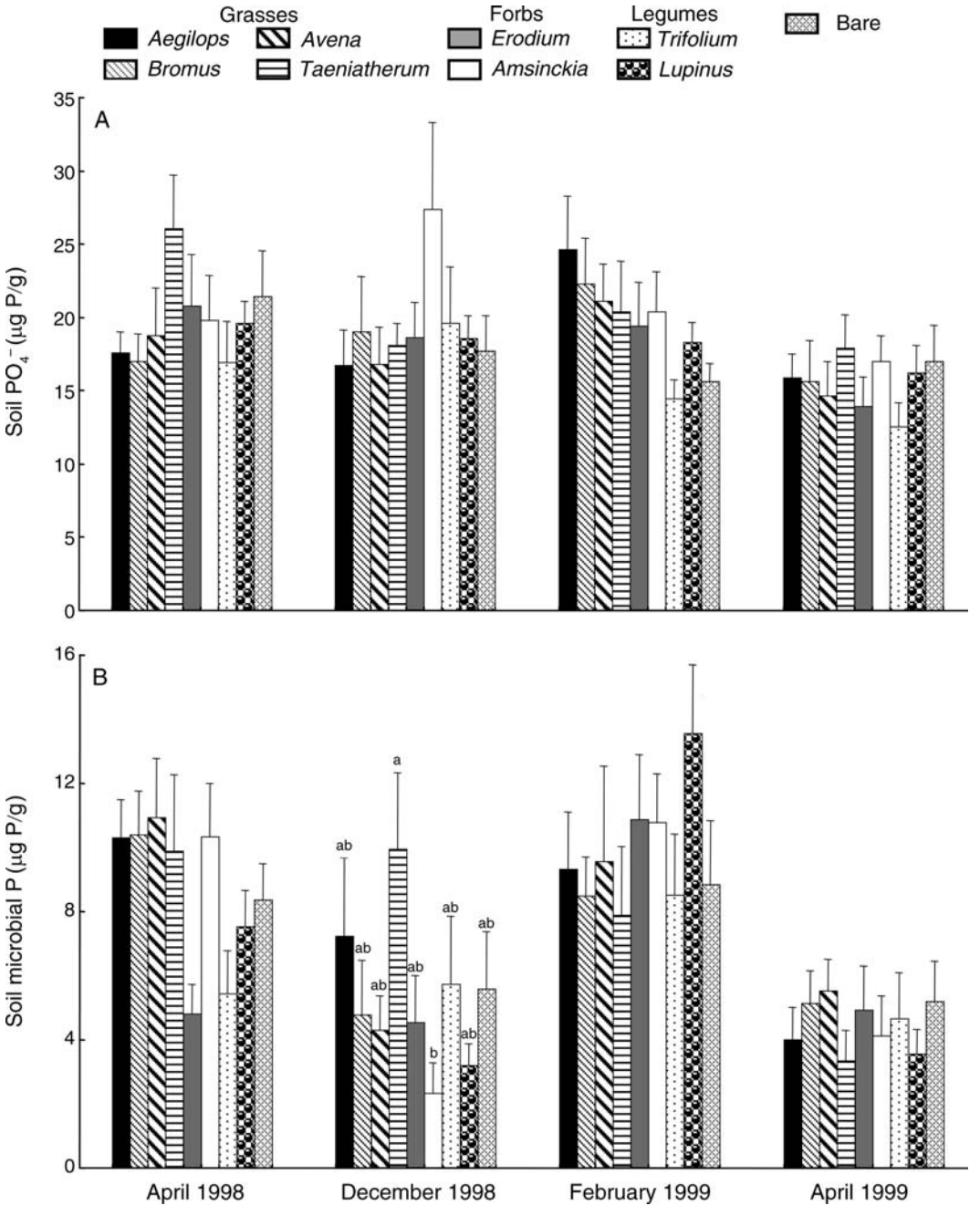

FIG. 5. Seasonal patterns of plant species effects on (A) soil PO_4^- and (B) microbial P per gram dry mass of soil. Data are means + SE ($n = 8$ replicates per treatment). Significant differences among species for each time point are designated by different letters (determined by a Tukey-Kramer post hoc test, $P \leq 0.10$).

DISCUSSION

Most studies investigating the effects of plant species on nutrient cycling have focused on litter chemistry as the key trait that determines patterns of species effects (Melillo et al. 1982, Taylor et al. 1989, Stump and Binkley 1993, Scott and Binkley 1997). However, a number of studies have demonstrated that traits other than litter chemistry can determine plant effects on N and P cycling (reviewed in Hobbie 1992, Eviner and Chapin 2003*a*). The goal of our study was to determine the roles of these different traits in mediating plant species effects on N and P cycling. The species studied in this experiment have been shown to differ significantly in all of the traits studied, with a 3.5-fold difference in litter C:N across species, a threefold difference in live C:N, a fivefold difference in plant biomass, a twofold

TABLE 3. Multiple regressions relating soil PO_4^- and microbial P with plant traits (see Table 1).

Response variable and explanatory variables	r^2	ss of factors	F	df	P	Relationship
December 1998						
Soil PO_4^-	0.88		18.5	2,7	0.005	
Max. soil temperature		64.9	33.8		0.0021	−
Litter C:N		23.1	12.0		0.02	+
Soil microbial P	0.77		8.2	2,7	0.03	
Live biomass		26.6	14.1		0.01	+
Litter C:N		9.5	5.0		0.07	+
February 1999						
Soil PO_4^-	0.8		24.0	1,7	0.003	
Litter C:N						+
Soil microbial P	0.5		6.1	1,7	0.05	
Bioavailable C						−
April 1999						
Soil PO_4^-	0.95		15.5	4,7	0.02	
Microbial P		16.1	50.8		0.006	−
Bioavailable C		6.4	20.3		0.02	−
Soil temperature (daily change)		4.4	11.03		13.8	−
Soil moisture		3.3	10.6		0.05	+
Soil microbial P	0.9		12.5	1,7	0.02	
Soil temperature (daily change)						−
April 1998						
Soil microbial P	0.95		18.3	2,7	0.008	
Bioavailable C		1325	124		0.001	−
Soil moisture		60	5.6		0.08	−

Notes: Rows for soil PO_4^- and soil microbial P show statistics, including r^2, for the overall model; rows for the significant factors include ss (when there is >1 significant factor) and the direction of the relationship. Results for soil PO_4^- in April 1998 are not shown because they are not significant.

difference in soil bioavailable C, a threefold difference in daily temperature variations, and a 30% difference in spring levels of soil moisture (Eviner 2004). Many of these traits varied seasonally, and these multiple traits were distributed independently from one another across species, so that plant species had distinct combinations of traits that changed over the growing season (Eviner 2004).

It is thus not surprising that, at any given time point, multiple traits often were better predictors of species effects on N and P cycling than any single trait. For example, in our study, two grass species (*Avena* and *Bromus*) had similar litter chemistry, as did two legume species (*Trifolium* and *Lupinus*). Based on litter chemistry alone, we would expect to see differences in N cycling between the grass and legume functional groups, but no differences between species within each functional group. However, at some time points, the species with the most similar litter chemistry had the least similar effects on net N mineralization and nitrification, probably due to species differences in soil bioavailable C. Even though bioavailable C represents a relatively small component of the total soil C pool, it can have large effects on N cycling (Flanagan and Van Cleve 1983, Vance and Chapin 2001), causing up to 10-fold differences in N cycling across species (Wedin and Pastor 1993). The sensitivity of N cycling to mechanisms other than litter chemistry was highlighted by the observation that plant species significantly differed in their effects on N cycling during the first growing season, before the presence of litter inputs. Similarly, in an experimental manipulation directly comparing plant species effects with and without litter, species effects on N cycling without litter feedback often differed from their effects in plots with litter.

Many other studies have also demonstrated that multiple traits need to be considered to account for species effects on ecosystems (Shock et al. 1983, Cheng and Coleman 1991, Bottner et al. 1999, Mack and D'Antonio 2003*a*, *b*). The importance of considering the roles of multiple plant traits becomes particularly critical for understanding seasonal changes in plant species effects. Over the growing season, we observed changes in the effects of plant species on N and P cycling, which can be accounted for by seasonal changes in plant traits (Eviner 2004), the relative importance of plant traits, and the impacts of a given trait (the latter two are probably related to seasonal changes in soil microbial activity and composition). For example, species effects on net N mineralization varied seasonally in lab incubations, as did the mechanisms that accounted for species effects. Potential rates of net N mineralization were correlated with bioavailable C in the fall, when plant belowground inputs are at their peak (Jackson et al. 1988), whereas litter chemistry became a stronger correlate in the winter and spring. Patterns of species effects on N mineralization in the field differed from lab incubations. Field rates of net mineralization were

strongly influenced by species effects on soil temperature in the fall and soil moisture in the spring, which are seasonally the main constraints on plant growth in California grasslands (George et al. 1988, Heady et al. 1991), and may similarly constrain net N mineralization. In contrast with mineralization patterns, potential rates of nitrification showed smaller seasonal variations in species patterns and in the traits that were correlated with these patterns. Patterns of net nitrification in lab vs. field incubations were very similar in the fall and winter, indicating that net nitrification may be less sensitive than mineralization to California's soil temperature regime. However, the large differences in species effects on net nitrification in lab vs. field incubations in April indicated that nitrification is sensitive to soil moisture.

The field incubations clearly demonstrate that species effects on soil microclimate can have strong impacts on N cycling. These microclimate effects not only impact nutrient cycling directly (Van Vuuren et al. 1992, Mack and D'Antonio 2003*a*), but also constrain the impact of other plant traits, such as litter or exudate inputs, which are important determinants of species effects on N cycling only when soil microclimate is not limiting (Meetenmeyer 1978, Burke 1989, Vinton and Burke 1997, Steltzer and Bowman 1998).

Plant species had stronger effects on net nitrification than they did on net mineralization, as seen in other studies (Templer et al. 2003). This may be because litter chemistry was frequently the dominant control over plant species effects on net nitrification, whereas species effects on net mineralization often were strongly influenced by other traits, such as bioavailable C and species effects on soil microclimate. In contrast, plant species effects on phosphorus were extremely weak, and phosphorus was controlled mainly by landscape factors, with P concentrations increasing downslope. Because P release from organic matter depends on demand for P (McGill and Cole 1981), the high availability of soil P at our site probably decreased P mineralization and the potential for plants to influence P dynamics, except through uptake. Other studies have shown that there often is not a clear link between P dynamics and biotic factors such as initial litter P (Blair 1988) or phosphatase activity (Gressel and McColl 1997).

As seen in other studies in California grasslands, species effects on soil N and P availability were not related (Hooper and Vitousek 1998), probably because N and P are influenced by different mechanisms (Eviner and Chapin 2003*b*).

Summary

Overall, this experiment demonstrated that: multiple plant traits best account for plant species effects on N and P cycling (Hypothesis 1); plant species effects on N and P cycling change seasonally, and multiple plant traits are particularly important to account for the seasonality of species effects on ecosystems (Hypothesis 2); soil temperature and moisture are critical determinants of plant species effects on N cycling, controlling seasonal patterns of field rates, and often masking the effects of litter chemistry (Hypothesis 3); and plant species can significantly alter N and P cycling, even in the absence of plant litter inputs (Hypothesis 4).

All together, this study clearly demonstrated the importance of considering the multiple mechanisms by which plant species influence ecosystems, highlighting the need for a mechanistic framework that predicts the ecosystem effects of plants based on multiple traits (Eviner and Chapin 2003*a*).

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