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EFFECTS OF PLANT COMPOSITION AND DIVERSITY ON NUTRIENT CYCLING

DAVID U. HOOPER[1] AND PETER M. VITOUSEK

Department of Biological Sciences, Stanford University, Stanford, California 94305-5020 USA

Abstract. We evaluated the effects of plant functional group richness on seasonal patterns of soil nitrogen and phosphorus cycling, using serpentine grassland in south San Jose, California. We established experimental plots with four functional types of plants: early-season annual forbs (E), late-season annual forbs (L), nitrogen-fixers (N), and perennial bunchgrasses (P). These groups differ in several traits relevant to nutrient cycling, including phenology, rooting depth, root : shoot ratio, size, and leaf C:N content. Two or three species of each group were planted in single functional group (SFG) treatments, and in two-, three-, and four-way combinations of functional groups. We analyzed available nutrient pool sizes, microbial biomass nitrogen and phosphorus, microbial nitrogen immobilization, nitrification rates, and leaching losses.

We used an index of "relative resource use" that incorporates the effects of plants on pool sizes of several depletable soil resources: inorganic nitrogen in all seasons, available phosphorus in all seasons, and water in the summer dry season. We found a significant positive relationship between increasing relative resource use (including both plant and microbial uptake) and increasing plant diversity. The increase in relative resource use results because different functional groups have their maximum effect on different resources in different seasons: E's dominate reduction of inorganic nitrogen pools in winter; L's have a stronger depletion of nitrogen in spring and a dominant reduction of water in summer; P's have a stronger nitrogen depletion in summer; N-fixers provide additional nitrogen in all seasons and have a significant phosphorus depletion in all seasons except fall. Single functional group treatments varied greatly in relative resource use; for example, the resource use index for the L treatment is as high as in the more diverse treatments.

We expected a reduction of leaching losses as functional group richness increased because of differences in rooting depth and seasonal activity among these groups. However, measurements of nitrate in soil water leached below the rooting zone indicated that, apart from a strong reduction in losses in all vegetated treatments compared to the bare treatment, there were no effects of increasing plant diversity. While some single functional group treatments differed (P $\leq$ L, N), more diverse treatments did not. Early- and late-season annuals, but not perennial bunchgrasses, had significant positive effects on microbial immobilization of nitrogen in short-term (24 h) ^{15}N experiments.

We conclude that: (1) total resource use, across many resource axes and including both plant and microbial effects, does increase with increasing plant diversity on a yearly timescale due to seasonal complementarity; (2) while the presence of vegetation has a large effect on ecosystem nitrogen retention, nitrogen leaching losses do not necessarily decrease with increasing functional group richness; (3) indirect effects of plants on microbial processes such as immobilization can equal or exceed direct effects of plant uptake on nutrient retention; and (4) plant composition (i.e., the identity of the groups present in treatments) in general explains much more about the measured nutrient cycling processes than does functional group richness alone (i.e., the number of groups present).

Key words: California; complementary resource use; diversity; ecosystem processes; leaching losses; microbial immobilization; nitrogen; nutrient cycling; phenology; phosphorus; plant composition; plant functional groups; serpentine grassland.

INTRODUCTION

While there is clear evidence that individual plant species can affect ecosystem processes such as nutrient cycling, the effects of plant diversity on nutrient cycling have received more discussion than experimental attention. Of the few experiments that have addressed this question, most have not separated effects of diversity per se from effects of species composition. For example, plant richness may influence nutrient cycling through complementary nutrient uptake: if, through niche differentiation, different species are able to acquire nutrients from different portions of the available

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[1] Present address: Integrative Biology, Room 3060, Valley Life Science Building, University of California, Berkeley, California 94720-3140 USA.

pool (either in space or time), total plant uptake may be greater and losses to leaching may be lower as diversity increases (Trenbath 1974, Bazzaz 1987, Vandermeer 1990). On the other hand, one or a few species may dominate nutrient uptake in more diverse communities, and have effects on the available pool similar to what they would have in monoculture (cf. Tilman 1988, Tilman and Wedin 1991*a*).

Plant species can influence ecosystem nutrient dynamics by a variety of mechanisms (Vitousek 1986, Vitousek and Walker 1989, Wedin and Tilman 1990, Van Cleve et al. 1991, Hobbie 1992, Chapin et al. 1995, Vinton and Burke 1995). To extend our knowledge of these species effects to those of plant diversity, we need to know how plant functional attributes combine in more diverse communities. Do traits of one species dominate, or do traits interact or average among species? How might their effects on ecosystem-level processes be modified by population- and community-level phenomena such as competition? And how do indirect effects of plants on nutrient cycling via microbial processes (e.g., nitrification and immobilization) interact with direct effects of plants (e.g., uptake of available nutrients) as plant diversity changes?

Intercropping experiments in both forestry and agriculture have investigated the effects of species mixtures on ecosystem nutrient dynamics (e.g., Vandermeer 1990, Binkley 1992, Morgan et al. 1992). These experiments have primarily focused on the influence of nitrogen-fixers in relatively low-diversity systems (2–3 species), and they commonly show increases in nitrogen availability to other species in the mixture (Vandermeer 1988, 1990). More complete leaf and litter cover in intercropped systems may reduce soil losses to erosion (Steiner 1982, Swift and Anderson 1993) and changes in soil fauna may affect litter decomposition and nutrient flux in mixed-species stands (Chapman et al. 1988, Blair et al. 1990, Williams 1994).

In nonagricultural systems, Ewel et al. (1991) found that soil nutrient pools differed little among diverse successional systems in tropical forests that differed in species composition and richness (succession, imitation succession, and enriched succession, all with >100 species). In contrast, significantly greater losses of cations and total soil nitrogen occurred in bare and monoculture treatments. Important factors reducing nutrient loss rates in diverse systems were increased plant uptake by native perennials vs. annual crops (due to greater root development and year-round uptake in perennials), and higher organic matter inputs to soil, which maintained soil cation exchange capacity (Berish and Ewel 1988, Ewel et al. 1991). Ewel et al. (1991) point out that differences may result from plant composition as much as plant diversity per se. For example, nutrient losses were substantially less under trees than under annuals even in monoculture crop rotations.

Grouping species by functional attributes is one way to address such questions mechanistically. While such functional classifications are not always clear-cut (Vitousek and Hooper 1993), they provide a useful step in addressing the importance of species diversity (Hobbie et al. 1993, Körner 1993). Previous research on the response of ecosystem processes to species richness has demonstrated intriguing patterns, but has not addressed mechanisms. For example, in microcosm experiments in growth chambers, decomposition of a common substrate (hay) and pool sizes of inorganic nitrogen, potassium, and phosphorus all differed significantly among treatments of different diversity, but not necessarily in order of species richness (Naeem et al. 1994, 1995). However, treatments were nested subsets of one another; accordingly, it is difficult to determine whether the observed responses are due to diversity per se (i.e., complementary resource use) or the functional traits of one or a few component species. In a large field experiment, Tilman et al. (1996) observed a general decline in soil nitrate concentrations within and below the rooting zone as diversity increased in experimental manipulations ranging from 1 to 24 species. Their results suggest that plant diversity may reduce nitrogen leaching losses and that the response asymptotes between 5 and 10 species. The decrease in soil nitrate may occur from nutrient use complementarity (Tilman et al. 1996), however the same pattern could result from dominance by one or a few species in more diverse treatments. Growing all species alone as well as in mixture would provide a direct test for the complementarity and dominance alternatives; at the same time, using functional groupings may give insight into the plant traits responsible for the underlying mechanisms.

Here we describe an experiment that examined how plant functional group richness and composition affect nutrient cycling in a serpentine grassland in California. Our objective was to assess how plant diversity affects integrative measures of nutrient dynamics (e.g., leaching losses and resource availability) as well as the mechanisms responsible for such effects. The experiment focused on both direct plant effects and on the importance of microbial immobilization for nutrient retention. We chose species from four groups defined by traits that are potentially relevant to nutrient cycling: (1) early-season annual forbs, (2) late-season annual forbs, (3) perennial bunchgrasses, and (4) nitrogen-fixers. Though defined here primarily by their phenology (except for N-fixers), these groups also differ in other characteristics relevant to nutrient retention and turnover, including rooting depth, root-to-shoot ratio, competitive ability on fertile and infertile sites, size, and foliage C/N ratio (Gulmon et al. 1983, Hobbs and Mooney 1985, Mooney et al. 1986, Chiariello 1989, Armstrong 1991).

Due to small plant size, predominantly annual phenology, and low soil organic matter pools, serpentine grasslands are a promising system in which to address

effects of diversity on ecosystem processes. They allow relatively easy experimental manipulations and reduce the chance that the effects of current species on ecosystem nutrient dynamics will be buffered by soil nutrient stocks. In addition to restrictions on plant growth due to regular summer drought in a Mediterranean-type climate, primary production in California serpentine grasslands is nutrient limited: N and P fertilization increases productivity, especially of exotic annual grasses that eventually dominate fertilized plots (Turitzin 1982, Koide et al. 1988, Huenneke et al. 1990). In this experiment, we focused on the effects of functional group composition and richness on nutrient cycling by growing each group alone as well as in more diverse combinations, and by making measurements throughout the year. While potentially important in natural ecosystems, the effects of other aspects of diversity, such as richness and evenness of species within functional groups, were not evaluated in this experiment.

Methods

Study site and experimental design

We established experimental plots on an area of serpentine soil in south San Jose, California, near the Kirby Canyon Landfill, operated by Waste Management, Inc. Hooper (*in press*) describes the experimental design in detail. To summarize, the experimental area was initially bare of vegetation and topsoil. Approximately 30 cm of serpentine topsoil from an on-site stockpile was graded over subsoil, and experimental plots were established in late autumn of 1991. Serpentine topsoil at this site is neutral (pH = 7.1 ± 0.03), has a relatively high clay content (% sand : % silt : % clay = 33:36:31; hydrometer method, DANR Analytical Laboratory, UC Davis, Davis, California), and is nutrient poor (total N = 1.29 ± 0.02 mg/g, total P = 0.200 ± 0.003 mg/g). By using revegetated plots with an initially uniform serpentine topsoil, we minimized differences in site quality and avoided confounding variation due to topography, soil depth, gopher disturbance, and harvester ant foraging (McNaughton 1968, Hobbs 1985, Hobbs and Mooney 1991, 1995).

The climate at this site is Mediterranean, with cool wet winters and a summer dry season extending from approximately May to October. Annual species germinate after the first significant rains in the fall. Early-season annuals and nitrogen-fixers set seed and senesce by early in the dry season (approximately May), whereas late-season annuals remain in rosette form through the wet season, then flower from June to October (Gulmon et al. 1983, Mooney et al. 1986, Chiariello 1989). Perennial bunchgrasses set seed in late May, then senesce aboveground during the dry season (Jackson and Roy 1986, 1989). Within each of these four functional groups, we chose two or three species that naturally occur in relatively high abundance in the area. These were: early-season annuals—*Lasthenia californica* DC. ex Lindley, *Microseris douglasii* (DC.) Schultz-Bip., and *Plantago erecta* (Morris); late-season annuals—*Hemizonia luzulaefolia* (DC.) ssp. *rudis* and *Lessingia micradenia* E. Greene var. *glabrata* (Keck) Ferris; perennial bunchgrasses—*Stipa pulchra* Hitchc. and *Sitanion jubatum* Smith; and nitrogen-fixers— *Lotus subpinnatus* Lag. and *Astragalus gambellianus* Sheldon. Nomenclature follows Thomas (1961), except for *Lessingia,* which follows Hickman (1993). We gathered seed for most species from several natural populations at Kirby Canyon, except seed for *Lasthenia californica,* which came from collections from serpentine populations at Jasper Ridge Biological Preserve.

The experimental design is a randomized complete block with 10 functional group mixes and six replicates, for a total of 60 plots. Plots were 1.5 × 1.5 m and separated by 0.5–1 m buffer strips. The treatments are as follows: bare (B); single functional group—E, L, P, N; two-group mixtures—EL, EP, LP; three-group mixture—ELP; four-group mixture—ELPN (where E = early-season annuals, L = late-season annuals, N = nitrogen-fixers, and P = perennial bunchgrasses). This gives a full factorial combination of E's, L's, and P's, plus two N-fixer treatments. Resources did not permit the full four-way factorial. We planted all treatments with a target aboveground biomass of ~200 g/m^2, which is similar to natural serpentine grassland in this area (McNaughton 1968, Turitzin 1982, Huenneke et al. 1990). We cut planting densities of single functional group treatments to one-half, one-third, or one-quarter in mixture treatments to maintain constant overall planting density. All species established successfully, and composition (though not density) was maintained by weeding. Results presented here are from soil samples collected during the second growing season (1992–1993), after treatments had fully established.

Seasonal soil sampling

Field sampling.—To assess seasonal dynamics of nutrient cycling, we sampled soil from all experimental treatments at four times of the year: in November 1992, 4 d after the first significant fall rains for that growing season; February 1993, during the wet midwinter growing season; May 1993, just after peak biomass of early seasonal annuals; and September 1993, after ~3.5 mo of no rain in the summer dry season. We collected three replicate cores of soil (5 cm diameter × 10 cm deep) from random locations in each plot. In May and September, these locations corresponded to sampling locations from biomass harvests (Hooper, *in press*). In other months, we clipped aboveground vegetation from each coring location before sampling. Soil cores were composited within each plot and manually homogenized in the laboratory. We removed large rocks and roots and subsampled for determination of soil moisture, inorganic N, microbial biomass N and P, available P, and microbial immobilization.

N extractions.—To measure inorganic nitrogen (am-

monium plus nitrate) pool sizes, we extracted ~20 g soil, oven-dry equivalent (o.d.e.), in 100 mL 0.5 mol/L K_2SO_4 immediately after soil mixing; all extractions were completed within 12 h of initial sampling. Extracts were frozen until analysis on an Alpkem RFA/2 autoanalyzer (Perstorp Analytical, Silverspring, Maryland) for ammonium (Alpkem RFA method number A303-S071-00) and nitrate (Alpkem RFA method number A303-S170-21). We sieved the remaining soil fraction to remove rocks >2 mm, and subtracted dry rock mass from the total soil mass before calculating NO_3^- and NH_4^+ concentrations on a microgram of N per gram of soil basis. All extractions described below follow the same basic procedure.

Microbial biomass and immobilization.—We determined microbial biomass nitrogen and phosphorus, as well as N immobilization, by the chloroform fumigation/direct extraction method (Brookes et al. 1982, Brookes et al. 1985, Davidson et al. 1989) on a subsample of soil incubated with ^{15}N for 24 h in the laboratory (Jackson et al. 1989, Davidson et al. 1991). We placed ~90 g (o.d.e.) subsamples of the composite soil into small ziplock bags to which we added 6.00 mL $(^{15}NH_4)_2SO_4$ solution at 99% atom excess, for a total addition of 90 μg ^{15}N (~1 μg ^{15}N/g soil). Soil and ^{15}N solutions were mixed thoroughly and incubated at room temperature for 24 h before extracting for initial nitrogen (as above) and phosphorus (15 g soil, o.d.e., in 100 mL 0.5 mol/L $NaHCO_3$, pH 8.5; Brookes et al. 1982). For fumigation, separate subsamples were wetted to 40–50% gravimetric soil moisture to avoid problems of different fumigation efficiencies at different soil water contents across seasons (Davidson et al. 1989). We fumigated with chloroform for 5 d before extracting for final nitrogen and phosphorus. Total nitrogen and phosphorus in Kjeldahl digests of extracts were analyzed for ammmonium (Alpkem RFA method number A303-S071-00) and orthophosphate (Alpkem RFA method number A303-S050-12) on the Alpkem autoanalyzer. ^{15}N enrichment in digested extracts was determined by diffusing ammonium onto acidified filter paper discs (Bremner 1960, MacKown et al. 1987). Discs were analyzed for their N isotopic content on a Europa Scientific Tracermass mass spectrometer in the laboratory of Dr. M. K. Firestone, University of California, Berkeley, California. We calculated microbial biomass nitrogen (or phosphorus) as the difference between final and initial total N (or total P, corrected for abiotic fixation; Brookes et al. 1982). Total phosphorus values from the initial extraction were used for estimates of total extractable phosphorus (TP_e). We calculated immobilization by the difference between final and initial ^{15}N pools in extracts ("^{15}N flush"). Microbial biomass N and ^{15}N were not corrected by a k_N factor because this has not been determined for serpentine soils. ^{15}N immobilization measurements were not attempted at the summer sampling because wetting the very dry soil to introduce the tracer can lead to large experimental artifacts.

Our ^{15}N flush values correlate quite strongly with immobilization rates calculated by the exponential model of Davidson et al. (1991). Pearson correlations of flush ^{15}N with ln(immobilization) are 0.81, 0.95, and 0.85 for November, February, and May, respectively. Because of very low NH_4^+ pools in some samples, calculations for many replicates could not be done by the exponential model. While the flush of ^{15}N does not yield actual immobilization rates (due to decreases in the ^{15}N pool over the course of the incubation and to differences in enrichment of the ammonium pool; Davidson et al. 1991), it gives a good relative comparison among treatments in this case. We will refer to it as "immobilization" in this paper.

Nitrification potential.—We determined nitrification potential in a 24-h aerobic slurry assay (Belser and Mays 1980), using no perchlorate to block transformation of NO_2^- to NO_3^- (Davidson et al. 1990). Initial tests determined that this was not necessary for consistent results as background levels of nitrate were relatively low (data not shown). Nitrification potential was calculated as the slope of the regression of nitrate concentration in the slurry (in micrograms of N per gram soil) against elapsed time. Any regressions that showed inconsistent accumulation of nitrate (R^2 less than ~0.80) were not included in data analysis.

Available P.—We determined available phosphorus using a laboratory assay (Saggar et al. 1990) with anion exchange membranes (Bio-Rex AG 1-X8 Anion Exchange Resin, Bio-Rad Laboratories, Richmond, California). We also incubated resin strips with standard solutions of known phosphate content, both within and substantially above the range of phosphate amounts generated by our soils, to be certain that the strips gave a linear response and never saturated; these conditions were satisfied in all samplings.

Total soil nitrogen and phosphorus.—To investigate whether functional group treatments led to differences in total soil nitrogen or phosphorus, we determined soil total nitrogen (TN) and total phosphorus (TP) from all plots at the beginning (December 1991, before any planting) and end (last full soil sampling, September 1993) of the experiment by using a modified Kjeldahl digest. We digested ~200 mg of soil with digest reagent (100 g K_2SO_4, 200 mL H_2SO_4, 0.29 g HgO) at 460°C for 2 h, diluted samples to 75 mL, and determined total solution nitrogen and phosphorus on the autoanalyzer, as above. Using a repeated-measures ANOVA (SYSTAT 1992), we tested for changes in total N and P, by treatment, between the two sampling dates. Because differences between years were small and inconsistent (data not shown), we averaged the values from the beginning and end of the experiment for each plot to obtain a single plot value for use in analysis of covariance when necessary.

Soil moisture.—In addition to measurements of grav-

imetric soil moisture in the top 10 cm during the regular seasonal sampling, we also measured soil moisture at three depths (0–10, 10–20, and 20–30 cm) in April and September 1993. Gravimetric soil moisture was calculated from mass loss after drying (105°C for 48 h). The April measurements are from a core tracer experiment that did not include N-fixer treatments (Hooper 1996). September measurements were from 5 cm diameter cores at each depth and include all treatments.

Leaching losses

To compare relative leaching losses among treatments, we placed porous cup lysimeters below the rooting zone (one per plot at 75 cm depth) in four of the six replicate blocks of each treatment (all six for the bare plots). Lysimeters were 10^5 Pa (1 bar) porous ceramic cups (Soil Moisture Equipment, Inc., Santa Barbara, California) attached to an 8-cm section of polyvinyl chloride (PVC) pipe (4.76 cm outer diameter, 4.13 cm inner diameter), sealed with a rubber stopper, with tubing leading to the soil surface. We backfilled the holes using silica flour around the lysimeter cup to maintain hydraulic contact with the surrounding soil, and included a bentonite layer with the subsoil to prevent excessive leaching in the lysimeter hole. Lysimeters were in place in the spring of 1992 and leachate collection began in January 1993 when all treatments had sufficient moisture at depth to obtain samples. We took samples monthly until April 1993 when most lysimeters were dry. Samples were stored in a cold room until they could be analyzed for NO_3^- and NH_4^+, as above. To buffer results from anomalously high concentrations in small volumes of leachate, we analyzed data both by nitrate concentrations (ammonium was always below detection) and by multiplying these concentrations by the volume of liquid collected to estimate the total pool of nitrate in the lysimeter leachate.

Litter quality

Senesced leaves and stems from bunchgrasses (*Sitanion* and *Stipa*) and senesced leaves and standing dead stalks (1 yr old) from late-season annuals (*Hemizonia* and *Lessingia*) were collected from the grassland near the experimental plots. Litter was mixed within functional group (and within litter type for L's), subsampled, ground on a Wiley Mill, and analyzed for total N and P by Kjeldahl digest. Lignin and cellulose were determined by an acid detergent method (Van Soest and Wine 1968). Early-season annuals (*Plantago*) were harvested from a separate experiment (Hooper 1996); seeds were separated from leaves, stems, and flowers. The latter were mixed, and analyzed as above. While the *Plantago* had not entirely senesced, separate analyses indicated that nitrogen and phosphorus concentrations did not differ significantly from completely dead plants from outside the experimental plots. We analyzed data on litter composition by one-way ANOVA with Tukey's post hoc comparisons among treatment means (SYSTAT 1992).

Calculations for relative resource use

To estimate total resource use in the vegetated treatments, we calculated the proportion that depletable resources (N, P, and water) were reduced in each treatment and in each season, relative to the bare treatments. We defined the "maximum reduction" as the difference between the bare treatment average and the average of whichever treatment had the lowest measured pool size in the season of interest. The "proportion of maximum reduction" for each plot is the difference between the measured value in that plot and the bare treatment average, divided by the maximum reduction:

$$\text{proportion of maximum reduction} = (B_{\text{avg}} - S)/(B_{\text{avg}} - V_{\text{min}})$$

where B_{avg} = average of all bare plots for the pool size of a given depletable resource (N, P, and water) in a given season; S = size of available pool of N, P, or water in each plot; and V_{min} = average value for the vegetated treatment with the greatest reduction in the level of that resource.

This was calculated for each vegetated plot for each season for inorganic N and available P (anion exchange resin) pool sizes and for September soil moisture (averaged across depth). An average value for inorganic N use was then calculated across all seasons and the same was done for available P use. Finally, these and the value for September soil moisture were averaged to give one index of proportion of total resources used (Relative Resource Use) for each plot. Interpreting this index to reflect resource use assumes that resource production rates are the same; furthermore, the index reflects microbial use (immobilization, denitrification, etc.) as well as plant uptake. Relative Resource Use was analyzed by regression against functional group richness (plus a categorical variable for block) using the General Linear Models and Nonlinear Curve Fitting functions in SYSTAT (SYSTAT 1992).

Statistics

Nutrient cycling data were analyzed both by composition and by level of richness (i.e., number of functional groups) using ANOVA, ANCOVA, and linear regression in SYSTAT (SYSTAT 1992). We used a priori contrasts for (1) comparison of bare plots with all vegetated treatments; (2) pairwise comparisons among single functional group treatments; and (3) comparison of mixtures with the average of their component single functional group treatments. Details of these analyses are given in the Appendix.

RESULTS

Available nutrients

Inorganic N pool sizes.—Variation of total inorganic N pools was greater among seasons than among func-

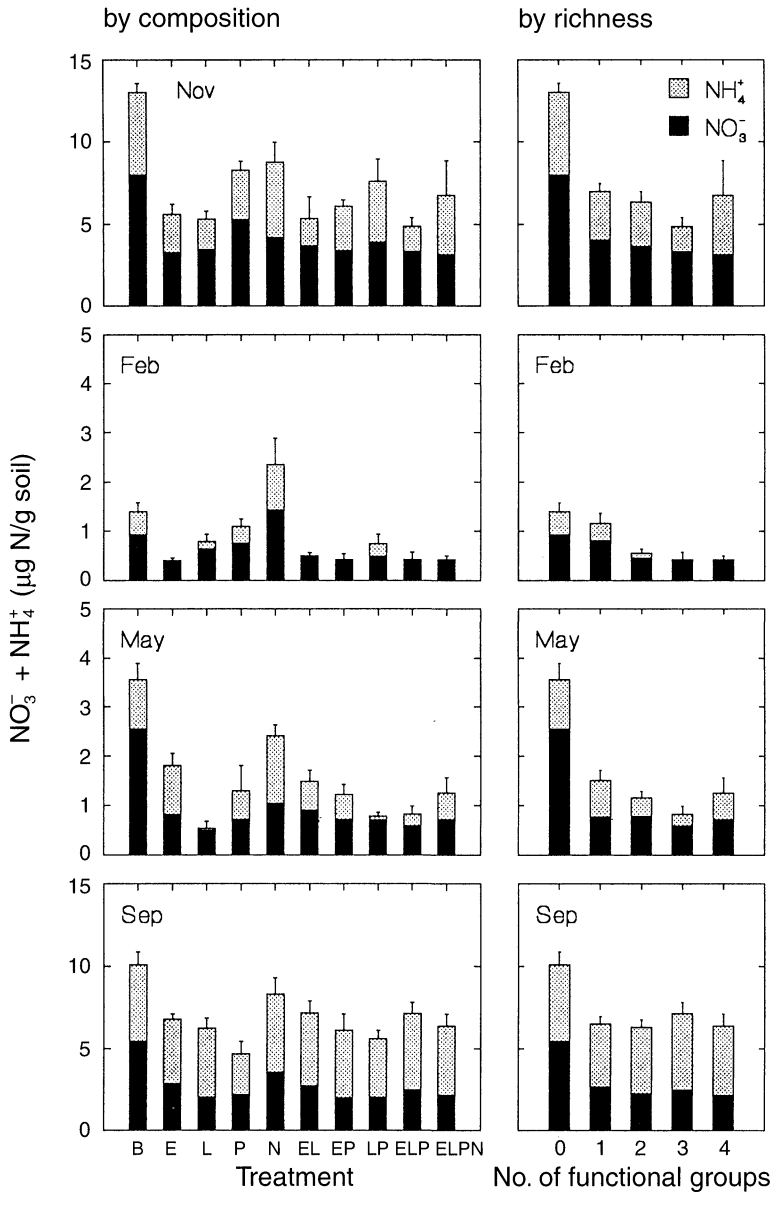

FIG. 1. Inorganic N pool sizes by month, plotted by functional group composition and by functional group richness. Functional group designations are B = bare soil, E = early-season annuals, L = late-season annuals, N = nitrogen-fixers, and P = perennial bunchgrasses. Individual plot values for the data graphed by richness are the same as those used for composition, only they are averaged over all treatments with the indicated number of functional groups: 0 = bare; 1 = E, L, P, and N; 2 = EL, EP, and LP; 3 = ELP; and 4 = ELPN. Values are nonadjusted cell means for nitrate (solid portion of bar) and ammonium (cross-hatched portion); error bars represent 1 SE of total inorganic N, $n = 6$. Note the differences in scale for different months. See Table 1 for statistics.

tional group treatments or levels of functional group richness (Fig. 1). All treatments had highest pool sizes in September (summer dry season) or November (just after the first fall rains); all had their lowest pool sizes in February (midwinter growing season); and all were intermediate in May (peak biomass of early-season annuals at the transition to the dry season). The strong effect of vegetation in reducing inorganic N pools persisted throughout all seasons (Table 1, Fig. 1). Either plants are directly depleting the available N pools relative to bare treatments and/or the presence of plants enables microbes to do so more effectively. Nitrate was the predominant form of inorganic N, except in September and except in the N treatment (Fig. 1). Ammonium was exceptionally low in all treatments in the midwinter (February) sampling, particularly in E single functional group (SFG) plots and E-containing mixtures.

Nitrogen-fixers alone had higher ammonium and nitrate pool sizes than the other single functional group

TABLE 1. Analysis of (co)variance results for initial inorganic nitrogen pools (0.5 mol/L K_2SO_4 extractable $NO_3^- + NH_4^+$; Fig. 1): (A) by composition; (B) by richness. AN(C)OVA was calculated by functional group composition in the plots, as well as by functional group richness (FG = number of functional groups, a categorical variable). Effects of richness were also analyzed by linear regression. See Appendix for details on statistics.

Analysis	Nov‡,§	Feb‡	May ‡,§	Sep§
A) By composition				
ANOVA				
r^2	0.652	0.753	0.722	0.642
Main effects and interactions‖¶				
E	+E < −E***	+E < −E***	...	...
L	+L < −L**	...	+L < −L***	...
P	...	...	+P < −P***	+P < −P**
N	...	...	...	+N < −N†
E × L	...	sat. (0.0138)	sat.*	sat. (0.032)
E × P	...	...	...	sat.*
L × P	sat.†	...	sat.**	sat.**
E × L × P	...	...	...	...
E × L × P × N	neg.†	...	neg.†	...
A priori comparisons‖				
Vegetation#	+v < −v***	+v < −v**	+v < −v***	+v < −v***
Among SFG's#				
E vs. L	...	E ≤ L (0.039)	L < E**	...
E vs. P	E ≤ P (0.022)	E < P**	...	P ≤ E (0.028)
E vs. N	E ≤ N (0.038)	E < N***	...	...
L vs. P	L ≤ P (0.018)	...	L ≤ P (0.030)	...
L vs. N	L ≤ N (0.032)	L < N***	L < N***	...
P vs. N	...	P < N**	P < N**	P < N**
Averaging#				
EL	...	...	...	...
EP	...	EP ≤ avg. (0.042)	...	...
LP	...	...	...	...
ELP	...	ELP ≤ avg. (0.021)	...	...
ELPN	...	ELPN < avg.**	...	...
B) By richness				
ANOVA				
r^2	0.478	0.404	0.499	0.523
Number of FG's	***	***	***	***
Linear trend††	***	***	***	***
Differences among levels of richness‡‡				
B	B > 1, 2, 3, 4	B > 2, 3, 4	B > 1, 2, 3, 4	B > 1, 2, 3, 4
1	...	1 > 2, 3; 1 ≥ 4	...	...
2	...	...	...	...
3	...	...	...	...
Regression				
r^2	0.313	0.355	0.267	0.257
Slope	−0.167***, B§§	−0.149***	−0.154***, B§§	−0.556*, B§§

Note: Functional group designations are B = bare soil, E = early-season annuals, L = late-season annuals, N = nitrogen-fixers, and P = perennial bunchgrasses.

‡ Variable was natural-log transformed to homogenize variance or to improve normality before analysis.

§ Pretreatment total soil nitrogen (TN) was used as a covariate.

‖Significance of ANOVA effects and a priori contrasts are given using familywide confidence intervals from Kimball's inequality: † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; nonsignificant trends, where $0.05 > P >$ Kimball-adjusted α, are indicated by ≥ or ≤ sign; numbers in parentheses are unadjusted P values; ... = not significant.

¶ Interactions are described as positive ("pos.": mixture effect greater than sum of functional group effects), negative ("neg.": mixture effect opposite to single functional group effects), or saturated ("sat": mixture effect same as single functional group effects).

A priori contrasts are for vegetation effects (mean of all vegetated treatments = bare treatment), pairwise comparisons among single functional group treatments (SFG), and averaging (mixture treatment = mean of component SFG treatments). See the Appendix for more details.

†† Linear trend in cell means, as tested by SYSTAT's contrast for first-order polynomial trends (SYSTAT 1992).

‡‡ Scheffé adjusted pairwise comparisons of means among levels of richness ($P < 0.05$): B = bare soil; 1 = average of all single functional group treatments; 2 = average of all two-group treatments; 3 = three-group treatment (ELP); and 4 = four-group treatment (ELPN).

§§ "B" indicates that regression by number of functional groups was significant due to the effects of the bare plots, i.e., when the regression analysis was repeated without data from the bare plots, the number of functional groups was not a significant predictor.

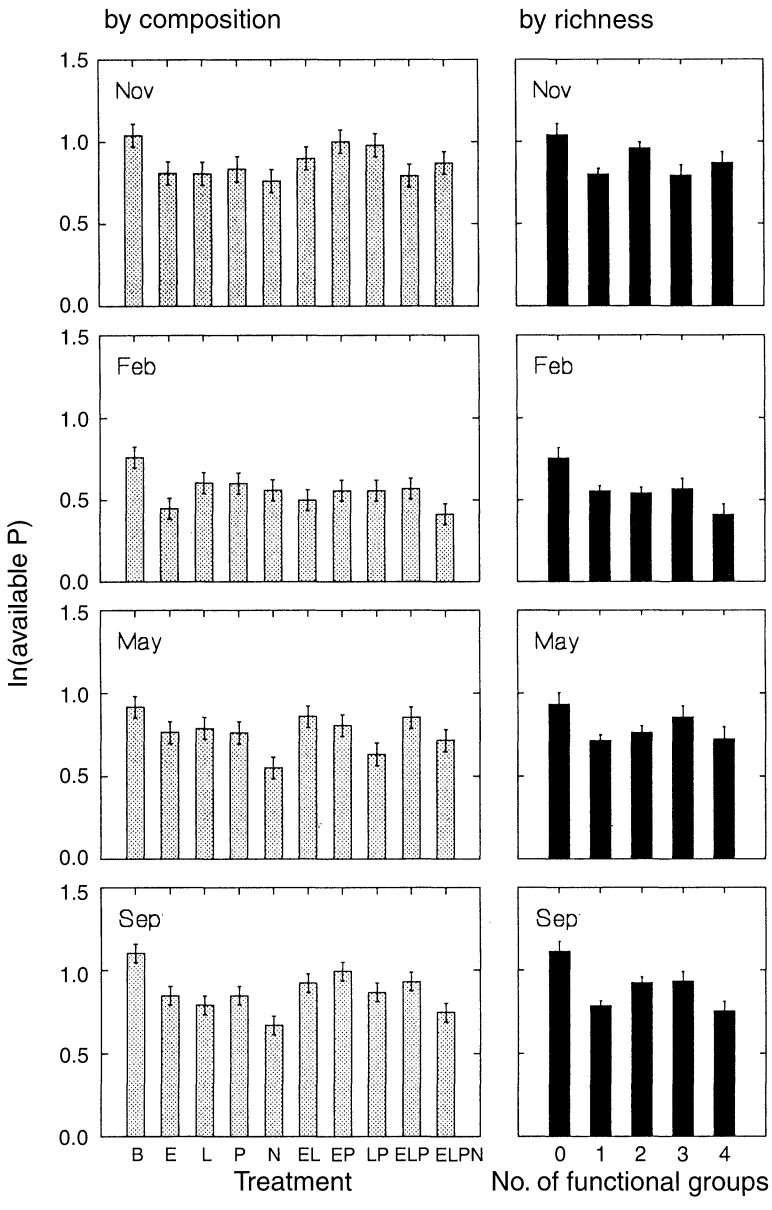

FIG. 2. Available phosphorus (anion exchange resin P) by month, plotted by functional group composition and richness. Treatment and diversity designations are as in Fig. 1. Values are adjusted means and standard errors ($n = 6$) from ANCOVA of natural-log transformed data using total soil phosphorus (TP) as a covariate. Log transformation done as $y = \ln(1 + y_0)$, where the original data (y_0) were in units of micrograms of P per gram soil. See Table 2 for statistics.

treatments in all seasons (Fig. 1), and this treatment was greater than or equal to the bare plots in February. The effects of N-fixers in the four-way mixture depended on season. In February, the additional nitrogen was presumably taken up by other species during this period of high demand. Though nitrogen-fixers reduced inorganic N pools below those of bare plots in November and May, they effectively increased available N pools in relation to the ELP treatment in those seasons (E × L × P × N interactions; Table 1, Fig. 1).

The different functional groups had maximum reduction of inorganic N pool sizes in different seasons (Table 1, Fig. 1). Although early and late season annuals had senesced by November, E and L single functional group treatments had lower (nonsignificant trend) pool sizes than perennial bunchgrasses or nitrogen-fixers at this time. The effect carried over into the more diverse plots, as indicated by the highly significant main effects (Table 1, Fig. 1). Functional group effects were not complementary, however: though E's, L's, and P's in single functional group treatments all reduced inorganic N pools below levels in the bare treatment, more diverse treatments showed no additional depletion in November (L × P interaction, Table

TABLE 2. ANCOVA results for available phosphorus (anion exchange resin P; Fig. 2): (A) by composition; (B) by richness. Labels and statistics are as in Table 1, except pretreatment total soil phosphorus was used as a covariate in all months.

Analysis	Nov‡	Feb‡	May	Sep‡
A) By composition				
ANCOVA				
r^2	0.570	0.504	0.550	0.672
Main effects and interactions				
E	...	+E ≤ −E (0.020)	...	...
L	...	...	...	...
P	...	...	...	...
N	...	+N < −N†	+N < −N**	+N < −N***
E × L	...	...	neg. (0.027)	...
E × P	...	sat. (0.038)	...	pos. (0.042)
L × P	...	...	...	...
E × L × P	...	...	...	...
E × L × P × N	sat. (0.013)	...	...	neg. (0.030)
A priori comparisons				
Vegetation	+v ≤ −v (0.022)	+v < −v*	+v ≤ −v (0.029)	+v < −v**
Among SFG's				
E vs. L	...	...	...	...
E vs. P	...	...	...	...
E vs. N	...	...	...	E ≥ N (0.029)
L vs. P	...	...	...	...
L vs. N	...	...	L ≥ N (0.022)	...
P vs. N	...	...	...	P ≥ N (0.029)
Averaging#				
EL	...	...	...	...
EP	EP ≥ avg. (0.041)	...	...	EP ≥ avg. (0.035)
LP	...	...	...	...
ELP	...	...	...	...
ELPN	...	...	...	...
B) By richness				
ANCOVA				
r^2	0.552	0.453	0.429	0.607
Number of FG's	**	**	†	***
Linear trend†,†	†	***	...	**
Differences among levels of richness‡,‡				
B	B > 1; B ≥ 3	B > 2, 4; B ≥ 1	B ≥ 1	B > 1, 4; B ≥ 2
1	...	...	...	...
2	2 > 1	...	...	2 > 1; 2 ≥ 4
3	...	...	...	...
Regression				
r^2	0.396	0.384	0.332	0.348
Slope	...	−0.055**, B	...	...

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
‡ Data were natural-log transformed to homogenize variance or to improve normality before analysis.

1). In February, early-season annuals reduced ammonium and nitrate to very low levels in the single functional group treatment and in all E-containing mixtures (E main effect, E mixtures lower than component averages [nonsignificant trend], and E × L interaction; Table 1). This pattern appears to reflect dominance by early-season annuals, in the sense of Tilman's R^*, where a competitively dominant species reduces pool sizes of available resources to lower levels than other species (Tilman 1988). As the growing season progressed, functional groups with later phenologies became more important in controlling inorganic N pools. In May, late-season annuals had the lowest pool sizes of the single functional group treatments, and in September, perennial bunchgrasses did (Table 1, Fig. 1). These groups also reduced pool sizes in more diverse treatments, though at those times mixtures did not differ from the average of their component functional groups.

The pattern of dominance by early-season annuals in February led to a significant decline in inorganic N pool sizes with increasing functional group richness in the diversity ANOVA and regression (Table 1, Fig. 1). However, while two- and three-way mixtures had lower available N pool sizes than did one-group treatments, on average, the E single functional group treatments were equally low, and the spread between single functional groups (even without including the N-fixers) was at least as great as the difference among levels of richness (Table 1, Fig. 1). Functional group composition

TABLE 3. ANCOVA results for total extractable phosphorus (TP_e; Fig. 3): (A) by composition, (B) by richness. Labels and statistics are as in Table 1, except pretreatment total soil phosphorus was used as a covariate in all months.

Analysis	Nov‡	Feb	May‡	Sep‡
A) By composition				
ANCOVA				
r^2	0.447	0.448	0.437	0.589
Main effects and interactions				
E	...	...	+E ≥ − E (0.040)	+E > −E*
L	...	...	...	+L > −L*
P	...	...	...	...
N	+N ≤ −N (0.018)	...	...	...
E × L	...	...	...	...
E × P	...	...	...	sat. (0.022)
L × P	...	...	...	...
E × L × P	...	...	...	...
E × L × P × N	...	...	...	...
A priori comparisons				
Vegetation	...	...	...	...
Among SFG's				
E vs. L	...	...	...	...
E vs. P	...	...	...	E ≥ P (0.047)
E vs. N	...	...	E ≥ N (0.035)	...
L vs. P	...	...	...	L > P†
L vs. N	...	...	...	L ≥ N (0.049)
P vs. N	...	...	...	...
Averaging#				
EL	...	...	EL ≥ avg. (0.020)	...
EP	...	...	...	EP ≥ avg. (0.014)
LP	...	...	...	...
ELP	...	...	...	ELP > avg.*
ELPN	...	ELPN ≤ avg. (0.042)	...	ELPN ≥ avg. (0.016)
B) By richness				
ANCOVA				
r^2	0.409	0.382	0.301	0.443
Number of FG's	*	...	...	**
Linear trend†,†	...	...	...	*
Differences among levels of richness‡,‡				
B	...	...	...	...
1	2 > 1	...	...	...
2	...	...	...	2 ≥ 1
3	...	...	...	3 > 1
Regression				
r^2	0.258	0.284	0.242	0.376
Slope	...	...	...	0.081**

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
‡ Data were natural-log transformed to homogenize variance or to improve normality before analysis.

explained 35–40% more of the variance in the data than did numbers of functional groups alone (Table 1). Significant decreases in inorganic N with increasing functional group richness also occurred in the other seasons. However, these trends resulted primarily from the bare plot (September) or hide even more significant differences among treatments of different composition within the same level of richness (November and May).

Anion exchange resin phosphorus.—Both season and plant composition had smaller effects on available phosphorus pools than they did on inorganic nitrogen pools sizes. As with inorganic N, available P pools were highest in summer and fall, lowest in the wet season, and intermediate in late spring (Fig. 2). While the presence of vegetation decreased available P below levels in bare plots (detectable as at least a nonsignificant trend in all seasons), this effect was much weaker than for available N (Table 2). N-fixers significantly reduced available P levels in February, May, and September ($P < 0.10$, 0.01, and 0.001, respectively).

Within seasons and within the top 10 cm of soil, these functional groups do not appear to partition available P in a complementary way. Regressions of available P against functional group richness were significant only in February, when the trend was due mostly to the difference between the bare and ELPN treatments (Table 2, Fig. 2). In November and September, two-way mixtures had significantly more available P than

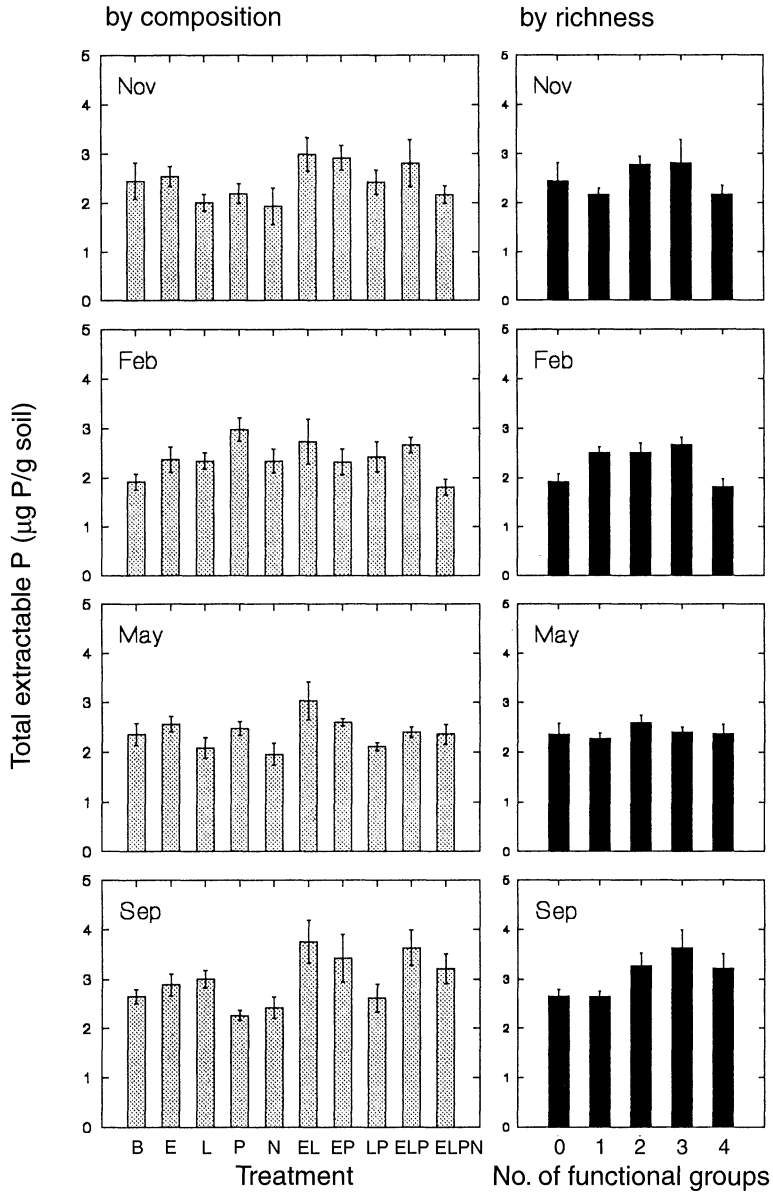

FIG. 3. Total bicarbonate-extractable phosphorus (TP_e; mean ± 1 SE, n = 6). See Table 3 for statistics.

did the single functional group plots. This could be because the SFG plots include the previously described depletion of P by N-fixers, while the two-way treatments included no N-fixers.

Bicarbonate extractable P.—Total extractable P (TP_e) was even less sensitive to composition, diversity, and season than was available P, particularly during the wet part of the growing season (Table 3, Fig. 3). In the summer dry season, however, the presence of annual species led to higher TP_e pools (E and L significant positive main effects, L > P, and ELP > average; Table 3). Furthermore, TP_e increased significantly with increasing functional group diversity in September. This pattern does not occur with available (anion exchange resin) P, so there are apparently pools of organically bound P, potentially available to microbial degradation, that increase through the dry season and correlate with plant functional group richness.

Water availability

Spring soil moisture.—Neither functional group composition nor richness influenced soil moisture in April, at the transition between wet and dry seasons. The primary effect was that vegetated plots, on average, had lower soil moisture than bare plots, by ~0.05 g H_2O/g soil in the upper 10 cm and by ~0.15 g H_2O/g soil in the 10–20 and 20–30 cm depths (data not shown).

Summer soil moisture.—Functional groups differed strongly in their effects on soil moisture during the dry

TABLE 4. ANOVA and regression results for soil moisture by depth in September (Fig. 4): (A) by composition, (B) by richness. Labels and statistics are as in Table 1.

Analysis	Depth (cm) 0–10	10–20	20–30
A) By composition			
ANOVA			
r^2	0.706	0.672	0.524
Main effects and interactions			
E	+E < −E*	...	...
L	+L < −L**	+L < −L***	+L < −L***
P	...	+P ≤ −P (0.045)	...
N	...	...	...
E × L	...	...	...
E × P	neg. (0.032)	neg. (0.007)	neg. (0.019)
L × P	...	...	...
E × L × P	neg. (0.006)	*neg.	neg. (0.010)
E × L × P × N	sat. (0.042)	...	...
A priori comparisons			
Vegetation	+v < −v†	...	...
Among SFG's			
E vs. L	E ≥ L (0.006)	E > L***	E > L**
E vs. P	P > E*	...	...
E vs. N	...	E > N†	E ≥ N (0.007)
L vs. P	P > L***	P > L†	P > L**
L vs. N	...	...	...
P vs. N	P > N***	...	P ≥ N (0.023)
Averaging			
EL	EL ≤ avg. (0.040)	EL < avg.**	EL ≤ avg. (0.027)
EP	EP < avg.†	EP < avg.***	EP ≤ avg. (0.013)
LP	LP ≤ avg. (0.033)	LP ≤ avg. (0.005)	...
ELP	ELP < avg.**	ELP < avg.**	ELP < avg.*
ELPN	ELPN ≤ avg. (0.007)	ELPN ≤ avg. (0.008)	...
B) By richness			
ANOVA			
r^2	0.388	0.430	0.213
Number of FG's	**	***	†
Linear trend	*	*	(0.063)
Differences among levels of richness			
B	B > 3	...	...
1	1 ≥ 3	1 > 2, 3	...
2	...	...	...
3	...	...	...
Regression			
r^2	0.355	0.242	0.124
Slope	−0.006***	−0.009**	−0.007*

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

season. While vegetation in general slightly reduced soil moisture in the surface layer (Table 4, Fig. 4), the perennial bunchgrasses actually had higher soil moisture than the bare plots in the upper 10 cm; both bunchgrasses and early-season annuals tended to have higher soil moisture in deeper layers. Strong functional group differences developed in lower layers, with E's and P's having the highest and L's the lowest soil moisture at all depths. The effect of the late-season annuals carried across all plots (highly significant L effect, Table 4). This appears to be a dominant effect of the late-season annuals because all L-containing mixture treatments were lower than the average of the component functional groups but not lower than L's alone. Early-season annuals and perennials interacted in the EP treatment: whereas the E and P single functional group treatments were actually wetter than bare plots, soil moisture in the EP mixture approached the low values seen in the L-containing treatments. At all depths soil moisture was lower than the average of both component groups (Table 4, Fig. 4).

Functional group richness explained substantially less variance than did plant composition, however, the effect of diversity remained significant even when bare plots were excluded from the regression (Table 4). This pattern is similar to results for inorganic N pool sizes. It resulted more from the dominant effect of the late-season annuals than from complementary effects of the individual functional groups.

These vegetation effects occurred at extremely low

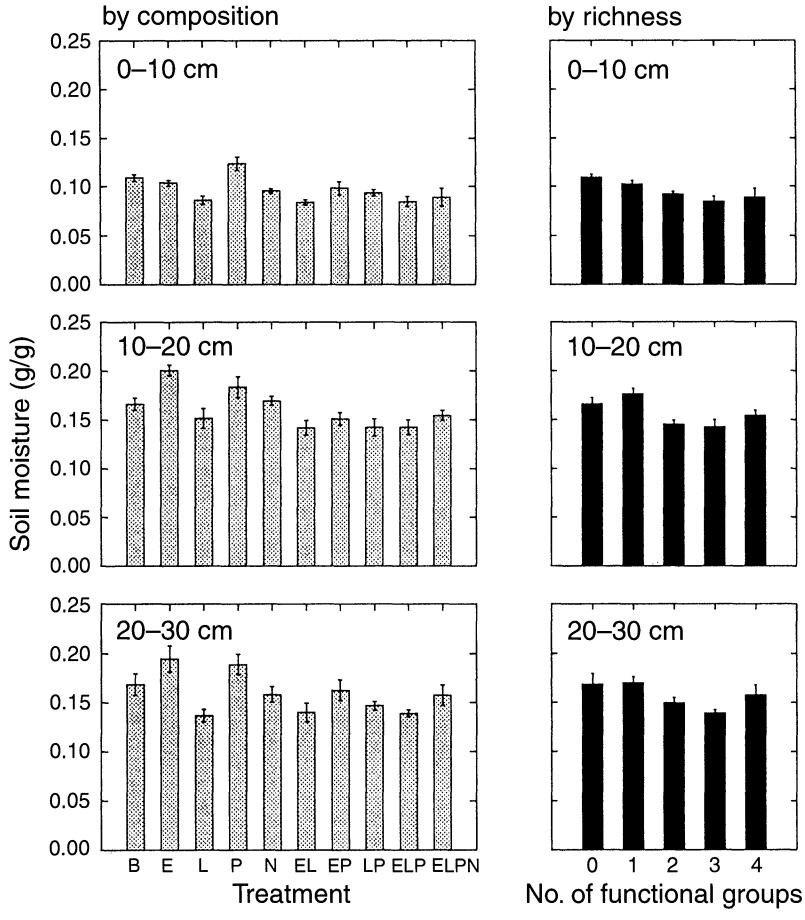

FIG. 4. Gravimetric soil moisture by depth, functional group composition, and richness for September 1993. Values are means and standard errors (n = 6) for each depth. See Table 4 for statistics.

soil moistures, well below what is commonly assumed to be plant available, at least for crops ($\Phi = -1.5$ MPa; Brady 1990). Moisture retention curves for both subsoil and topsoil (data not shown) gave soil moistures of 23.7 ± 0.3 and 29.9 ± 0.4%, respectively, at −1.5 MPa. Late-season annuals reduced soil moisture to ~9% in 0–10 cm layers and 14% in 10–20 and 20–30 cm layers.

Relative resource use

Average total resource use, relative to the bare treatments, showed a clear, highly significant, saturating increase with increasing diversity (Fig. 5), as indicated by the fitted curve and mean values for each level of diversity ($y = 0.374 + 0.292 \ln(FG)$, $R^2 = 0.203$, $P < 0.001$). Because N-fixers also substantially altered nitrogen supply, the relatively low values for the ELPN treatment reflect N inputs (Fig. 1) as well as plant and microbial uptake. When only treatments without N-fixers are plotted, the relationship was linear from one to three functional groups (2–7 species), and again highly significant ($y = 0.124 + 0.231FG$, $R^2 = 0.313$, $P = 0.001$; Fig. 5). Despite the broad pattern of increase with diversity, however, some functional groups (e.g., L's) equaled the effects of the more diverse treatments by this index. In addition to their dominant effect on soil moisture, L's also had strong effects on inorganic N pool sizes in November, February, and May (though not as strong as E's in February; Fig. 1), which contributed to their high relative resource use. There was no correlation between relative resource use and 1993 aboveground production (Pearson $r = -0.195$, $P = 0.16$; Hooper, *in press*).

Leaching losses

Increasing diversity did not reduce nitrogen loss by leaching in this experiment. While individual functional groups differed (P ≤ L, N; nonsignificant trends), none of mixture treatments differed from the averages of their component functional groups and there was no trend for decreased losses with increased diversity, except that caused by the bare treatments (Table 5, Fig. 6). Functional group identity explains more of the variance in leaching losses than does richness of functional groups alone, presumably due to differences among single functional group treatments. Nitrate was the primary form of nitrogen leached from these plots; am-

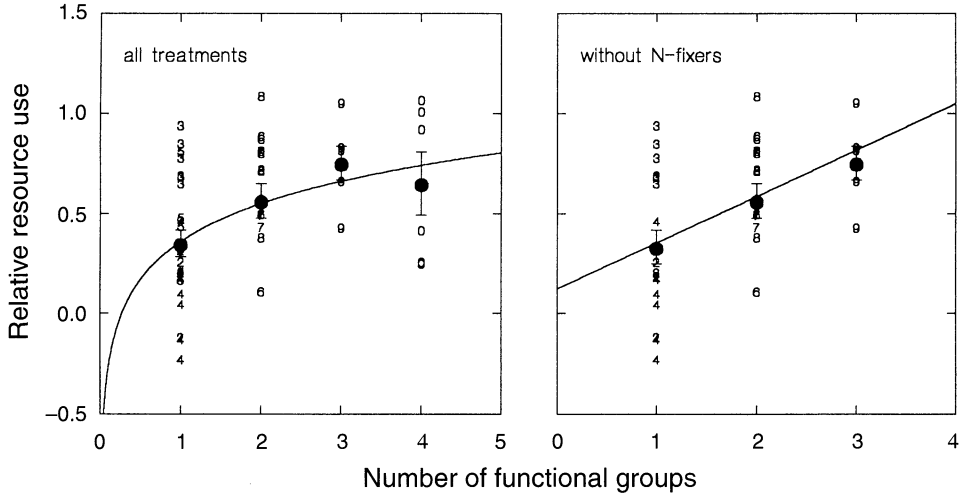

FIG. 5. Relative resource use vs. functional group richness. Relative resource use is the drawdown of pool sizes of inorganic nitrogen, available phosphorus, and soil moisture relative to the difference between bare plots and the vegetated treatment with the lowest pool size for each of those resources. Values for each plot and each resource are averaged over the year (nitrogen and phosphorus) or over soil depth in September for soil moisture. The yearly averages for nitrogen and phosphorus and the value for September soil moisture are averaged again to provide an unweighted index of resource use. See *Methods* for more details. Because N-fixers presumably alter nitrogen supply as well as demand, functions are plotted both with (all treatments) and without N-fixer treatments (N and ELPN). Numbers identify plot composition. One-way combination of functional groups: 2 = E, 3 = L, 4 = P, 5 = N; two-way: 6 = EL, 7 = EP, 8 = LP; three-way: 9 = ELP; four-way: 0 = ELPN. Lines are nonlinear logarithmic regression (all treatments) or linear least squares regression (without N-fixers; SYSTAT 1992). Circles with error bars are means ± 1 SE for each level of richness.

monium was never above detection limit (we did not measure dissolved organic N). Almost all of the leaching losses occurred in January, the first month in which collectable amounts of water reached the lysimeters at 75 cm depth. The only exceptions to this were the nitrogen-fixer and bare treatments, in which detectable concentrations of nitrate continued to leach until March and April, respectively (Fig. 6c). Plant uptake is presumably responsible for the decrease in losses from January to February (Fig. 6), when even the relatively shallow roots of early-season annuals reduce leaching of nitrate to barely detectable levels, similar to their effects on soil inorganic N pools (Fig. 1).

Microbial dynamics

Microbial biomass nitrogen (MBN).—Functional groups differed in their effects on microbial biomass nitrogen. The presence of annuals led to significantly higher MBN in all seasons except summer (positive L main effects in November, February, and May, and the E treatment is similar to the L SFG in all months; Table 6, Fig. 7). On the other hand, the P treatment had the lowest MBN of all single functional group treatments in November and February. In addition to these composition effects, plant diversity influenced MBN in two different ways. First, the effect of the EP mixture was not predictable from the E and P single functional group treatments: in the EP treatment, the positive effect of the annuals disappeared and MBN was as low as the B and P treatments in all months (EP < average in November and September; negative ExP interactions in November, May, and September; Table 6, Fig. 7). Second, MBN was lowest in most treatments in the summer, but despite this, functional group richness had a positive effect on MBN in both May and September, which was independent of bare treatments.

Microbial biomass P.—In most seasons, functional group treatments had little influence on microbial biomass phosphorus (MBP), whether focusing on functional group composition or richness (Table 7, Fig. 8). In September, MBP increased significantly with increasing functional group richness (similar to TP_e). This is also the season that has the lowest overall values of MBP in all treatments, as with microbial biomass N.

Immobilization.—The effects of the functional group treatments on microbial nitrogen immobilization were similar to their effects on MBN (Fig. 9). Annuals had a positive effect on immobilization in all months measured (significant E main effects in November and February, L main effects in February and May), whereas P's had negative effects in mixtures with annuals in the fall (trend toward E × L × P interaction; Table 8). In February, ^{15}N immobilization reached a maximum whether E's, L's, or both were present in any combination (E × L interaction). The strong positive effects of annuals and the negative effects of perennials on microbial immobilization partially offset differences among these groups in plant uptake in November. Because annuals are senescent in this season, plant uptake by E's and L's was negligible, yet inorganic N pool sizes were equal to or lower than P's (Table 1, Fig. 1) and leaching losses were only slightly higher than P's

TABLE 5. ANOVA results for lysimeter leachate (Fig. 6). Labels and statistics are as in Table 1.

Analysis	Total NO_3^- lost	January $[NO_3^-]$
A) By composition		
ANOVA		
r^2	0.650	0.681
Main effects and interactions		
E	...	...
L	...	...
P	+P ≤ −P (0.019)	...
N	...	...
E × L	...	...
E × P	sat.†	sat. (0.008)
L × P	...	sat. (0.022)
E × L × P	(0.050)	...
E × L × P × N	...	...
A priori comparisons		
Vegetation	+v < −v**	+v < −v*
Among SFG's		
E vs. L	...	...
E vs. P	...	...
E vs. N	...	E ≤ N (0.025)
L vs. P	P ≤ L (0.039)	...
L vs. N	...	...
P vs. N	P ≤ N (0.008)	P < N*
Averaging	No significant differences	
B) By richness		
ANOVA		
r^2	0.490	0.460
Number of FG's	**	*
Linear trend	*	*
Differences among levels of richness		
B	B > 1, 2, 3	B > 1, 2
1	...	...
2	...	...
3	...	...
Regression		
r^2	0.278	0.337
Slope	−0.677*, B	−0.271*, B

Note: Data were natural-log transformed to homogenize variance or to improve normality before analysis.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(Table 8, Fig. 6). Plant diversity effects on immobilization were also similar to those on MBN in that functional group richness had a stronger positive effect on immobilization as the soils dried out in May (values from this sampling are not directly comparable to those of the February and November samplings because in May the prefumigation incubation was 2 d, rather than 24 h). There were significant positive diversity effects in February, but these were driven predominantly by differences among SFG treatments.

Nitrification.—Functional group treatments had little effect on nitrification potential (Table 9, Fig. 10). In general, nitrifier populations increased through the growing season from November to February to May. In November, the presence of vegetation significantly reduced nitrification potential relative to the bare treatment, except for an anomalously high value in the LP treatment (L × P interaction, LP > average). In February and May, we detected no significant effects of plant presence, composition, or diversity (Table 9, Fig. 10). From this data, it appears that plant effects on nitrification rates were minimal.

Litter quality

While litter quality of the functional groups differed, there was no clear differentiation among groups in terms of "high-quality" and " low-quality" litter (Table 10). For example, while P's have lower %N in aboveground litter and roots, they also have lower lignin in shoots, such that lignin/N ratios do not differ greatly from other groups. Early-season annuals were distinguished by their very high phosphorus concentrations, but they also have relatively high lignin/N ratios. Within the L functional group, litter nutrient and lignin composition varied dramatically between leaves and stalks. Rosette leaves of late-season annuals had high nitrogen concentrations and low lignin/N and

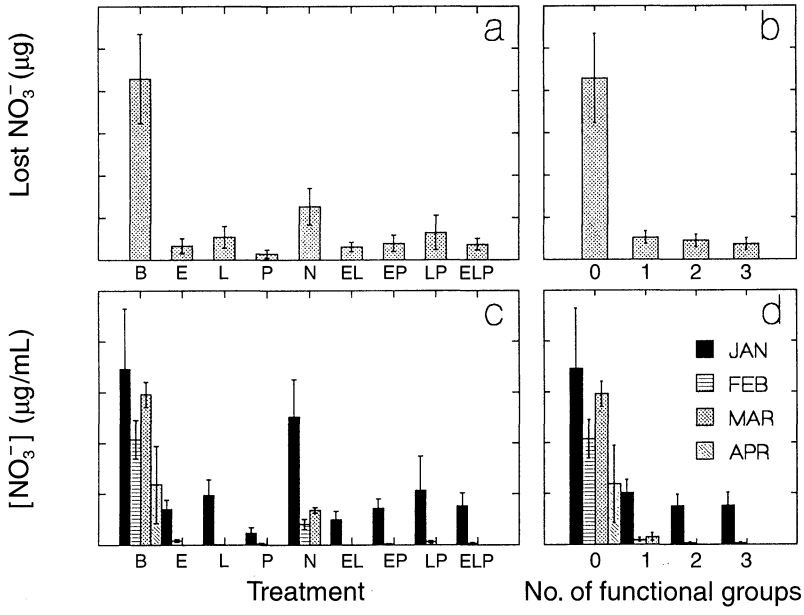

FIG. 6. Leaching losses by functional group composition (a, c) and richness (b, d). Losses (mean ± 1 SE, $n = 4$) are graphed by total micrograms of nitrate (volume × concentration) collected in lysimeters placed at 75 cm depth (a, b). Monthly nitrate concentrations in those lysimeters are also shown (c, d). For most treatments except bare (B) and N-fixers alone (N), nitrate was undetectable in all months except January. Because of malfunctioning lysimeters in the ELPN plots during the January sampling, when the highest concentrations of nitrate were detected, these data were excluded. See Table 5 for statistics.

lignin/P ratios, whereas stalk litter had very high cellulose and lignin/P.

DISCUSSION

Four main points emerge from our results: (1) relative resource use, across many resource axes and including both plant and microbial effects, increased with increasing plant diversity over the course of the entire year; (2) while the presence of vegetation had a large effect on ecosystem nitrogen retention, nitrogen leaching losses did not decrease with increasing plant diversity; (3) the indirect effects of plants on microbial processes can equal or exceed direct effects of plant uptake on nutrient retention; and (4) plant composition explained much more about the measured nutrient cycling processes than did the number of functional groups alone.

Relative resource use

The increase in relative resource use as diversity increased resulted primarily from seasonal complementarity among functional groups, and from dominance of different resources by different groups, rather than from spatial complementarity within seasons. The functional groups depleted pool sizes of inorganic nitrogen in a pattern that corresponds with expected timing of plant uptake, with the maximum effect of E's in February, L's in May, and P's in September (Woodmansee and Duncan 1980, Gulmon et al. 1983, Jackson and Roy 1986, Chiariello 1989). Within seasons, however, competition had a large effect on available nitrogen pools, soil moisture, and interactions among functional groups, despite differences in rooting depth among these groups. The pattern of inorganic N in February and of soil moisture in September is similar to that expected under Tilman's R^* hypothesis, in which the best competitor reduces resources to the lowest level (Tilman 1988, Tilman and Wedin 1991*b*). Dominance by E's for inorganic N helps explain previously described patterns of biomass, in which early-season annuals greatly reduce growth of perennial bunchgrasses (Hooper, *in press*). The results for inorganic N are also consistent with Gulmon et al. (1983) in showing that *Plantago erecta* is a strong competitor for nitrogen, and with predictions by Berendse (1979, 1982) that deeply and shallowly rooted species can coexist if the more shallowly rooted one(s) are the best competitors. In this case, the deeply rooted perennials may be getting nutrients at different times of the year instead of (or as well as) at different depths (Jackson and Roy 1986).

The seasonal climate pattern at Kirby Canyon leads to shifting resource limitations: nutrients limit growth in midwinter, while water limits growth in late spring and summer (Turitzin 1982, Woodmansee and Duncan 1982, Gulmon et al. 1983, Koide et al. 1988, Chiariello 1989, Huenneke et al. 1990). While early-season annuals dominate in the former case (Fig. 1), late-season annuals dominate in the latter (Fig. 4). L's depend on deep water in late spring and summer to complete flow-

TABLE 6. ANCOVA results for microbial biomass nitrogen (MBN; Fig. 7). Labels and statistics are as in Table 1.

Analysis	Nov	Feb	May‡	Sep
A) By composition				
ANCOVA				
r^2	0.717	0.734	0.698	0.616
Main effects and interactions				
E	...	...	...	...
L	+L > −L†	+L ≥ −L (0.012)	+L ≥ −L (0.012)	...
P	...	...	...	...
N	+N ≥ −N (0.036)	...	+N ≥ −N (0.050)	...
E × L	...	...	...	...
E × P	neg. (0.013)	...	neg. (0.009)	neg. (0.009)
L × P	...	...	...	...
E × L × P	...	...	...	...
E × L × P × N	...	...	...	...
A priori comparisons				
Vegetation	...	...	+v > −v†	+v ≥ −v (0.015)
Among SFG's	P < N†	...	...	...
Averaging				
EL	...	...	...	...
EP	EP ≤ avg. (0.016)	...	...	EP ≤ avg. (0.035)
LP	...	...	LP ≥ avg. (0.009)	...
ELP	...	...	...	...
ELPN	...	...	ELPN ≥ avg. (0.023)	ELPN ≥ avg. (0.029)
B) By richness				
ANCOVA				
r^2	0.547	0.676	0.640	0.545
Number of FG's	...	...	*	*
Linear trend	...	...	***	**
Differences among levels of richness	...	...	B < 2, 4	B < 4
Regression				
r^2	0.519	0.658	0.618	0.517
Slope	...	...	0.097**	1.016**

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
‡ Data were natural-log transformed to homogenize variance or to improve normality before analysis.

ering and seed set, effectively partitioning this resource in time and space with the early-season annuals (Gulmon et al. 1983, Hobbs and Mooney 1985, Armstrong and Huenneke 1992). However, because perennial bunchgrasses also depend on late spring and summer moisture (Jackson and Roy 1986), the strong L effect on soil moisture in all layers helps to explain the high over-summer mortality of bunchgrasses in LP treatments (Hooper, *in press*). *Hemizonia* has also been shown to have strong competitive effects on oak seedlings due to soil water depletion to −6.0 MPa (Gordon and Rice 1993). While removing late-season annuals from the system would have a relatively minor effect on soil water availability in one case (compare EP and ELP treatments, Fig. 4), they dominate soil moisture in every other mixture of which they are a part. This illustrates the difficulty of trying to deduce whether species or functional groups in a diverse system are "redundant" (Lawton 1994, Lawton and Brown 1993) from manipulations of one or two under a single set of environmental conditions.

The significant effect of N-fixers on available phosphorus pools fits with the demand of N-fixers for this nutrient (Pate 1986, Vitousek and Howarth 1991) and with the positive response of *Lotus subpinnatus* and other N-fixers to phosphorus fertilization in previous studies in this system (Hobbs et al. 1988, Koide et al. 1988). No differences emerged among the other functional groups. Although fertilization studies have shown that P can limit (or co-limit with N) productivity in the serpentine grassland, generally only certain groups respond, particularly non-native annual grasses (Turitzin 1982, Koide et al. 1988, Huenneke et al. 1990).

It is possible that if we looked at more resources, e.g., cations, we would see an even stronger response of relative resource use to functional group richness. Cation imbalance is presumed to restrict species composition in the serpentine in general (Kruckeberg 1984, Baker et al. 1992) and cation addition has been shown to enhance productivity in nearby serpentine grassland (Huenneke et al. 1990, but see also Turitzin 1982, Koide et al. 1988). Ewel et al. (1991) also saw large differences among monoculture and high-diversity treatments in cation retention and acid saturation of cation exchange sites.

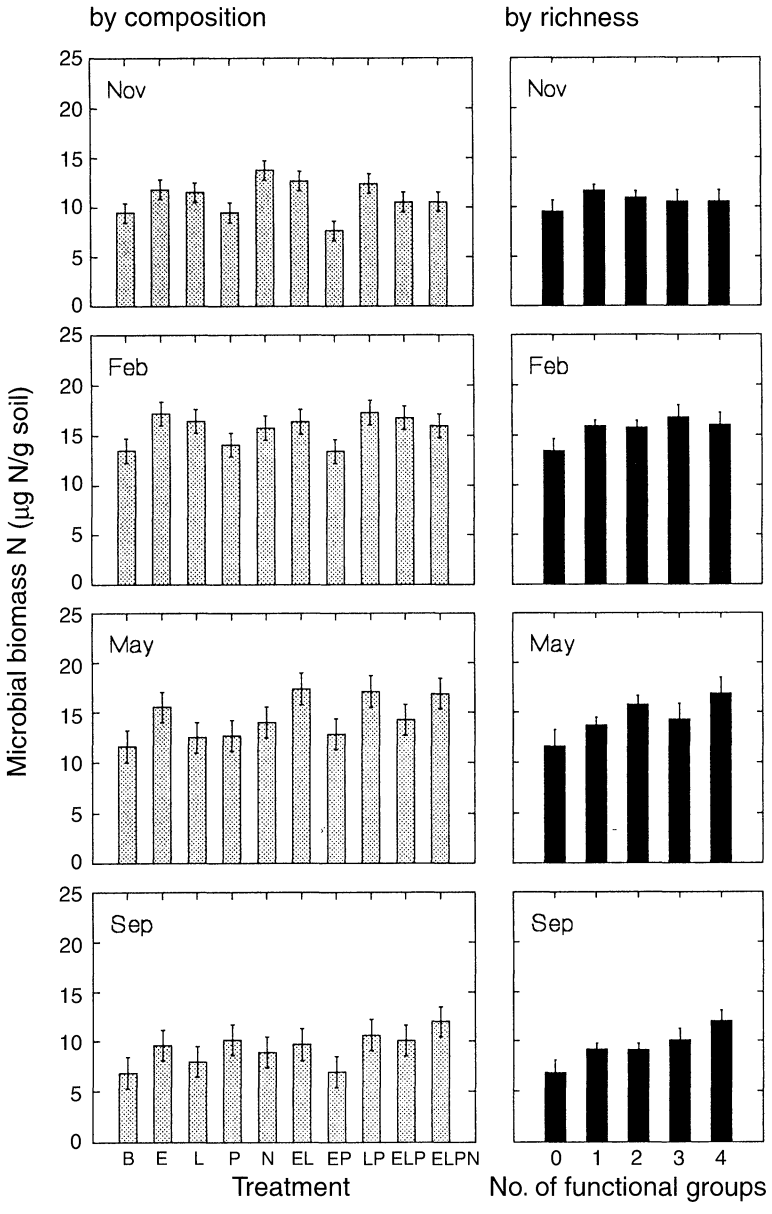

FIG. 7. Microbial biomass nitrogen (chloroform-labile N) plotted by functional group composition and richness. Values are adjusted means and standard errors (n = 6) from ANCOVA using TN as a covariate. See Table 6 for statistics.

In addition to plant uptake, microbial responses also contribute to functional group effects on resource pools. For example, we had anticipated that bunchgrasses would have the lowest pool sizes of nitrogen in November because their perennial root system would be active and taking up available N as soon as water was available (Jackson and Roy 1986, Jackson et al. 1988). However, indirect effects of E's and L's on microbial immobilization (Fig. 9) may have contributed to the greater reduction in inorganic N pool sizes in treatments with annuals (Fig. 1) because E's and L's had senesced by November. It is likely that these indirect effects on microbial immobilization contributed to patterns of pool size depletion in other seasons as well (Fig. 9; Wedin and Tilman 1990)(see *Leaching losses and indirect plant effects,* below).

Despite the general trend of increasing relative resource use with increasing diversity, there is much variability within levels of functional group richness. Of the single functional group treatments, the perennial bunchgrasses had the smallest overall effect on resource pool sizes, whereas the late-season annuals alone had as great an effect on relative resource use as any of the mixtures. This degree of variation within levels of richness is similar to the productivity response to diversity observed in pot experiments by Naeem et al. (1995). In an experimental diversity gradient in the field (Tilman et al. 1996), nitrate pool sizes both within

TABLE 7. ANCOVA results for microbial biomass phosphorus (MBP; Fig. 8): (A) by composition, (B) by richness. Labels and statistics are as in Table 1, except pretreatment total soil phosphorus was used as a covariate in all months.

Analysis	Nov	Feb	May	Sep
A) By composition				
ANCOVA				
r^2	0.629	0.506	0.498	0.503
Main effects and interactions		No significant main effects in any season		
E × L	...	...	...	...
E × P	...	...	neg. (0.028)	sat. (0.030)
L × P	neg. (0.033)	...	...	...
E × L × P	...	...	...	...
E × L × P × N	...	...	...	...
A priori comparisons				
Vegetation	...	...	...	...
Among SFG's		No significant differences		
Averaging				
EL	...	...	...	...
EP	EP ≤ avg. (0.026)	...	...	...
LP	LP ≥ avg. (0.022)	...	LP > avg.*	LP ≥ avg. (0.028)
ELP	...	...	...	...
ELPN	...	...	...	...
B) By richness				
ANCOVA				
r^2	0.493	0.468	0.374	0.404
Number of FG's	...	...	...	†
Linear trend	...	...	...	*
Differences among levels of richness		No significant pairwise comparisons		
Regression				
r^2	0.486	0.459	0.325	0.349
Slope	...	...	...	0.204*

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

and below the rooting zone declined with diversity, and lower levels of diversity had higher variance in nitrate pool sizes, suggesting a pattern like the one seen in this experiment. The characteristics of certain species or functional groups may allow them to exploit resources as efficiently as more diverse communities, and potentially to dominate ecosystem response in more diverse assemblages. Indeed, one effect of diversity may be that it increases the likelihood that such an "effective" species will be present (see *Composition and diversity effects,* below).

As plant diversity increased, relative resource use increased but aboveground production did not (Hooper, *in press*). There are three reasons why productivity may not correlate with our index of resource use. First, "use" in this index includes microbial as well as plant nutrient uptake; some microbial "use," e.g., denitrification or immobilization in the short term, may actually work against plant productivity. Second, if functional group effects on resource pool sizes occur more by changing magnitudes of loss or supply rates than by changing plant or microbial uptake (N-fixers with nitrogen, potentially bunchgrasses with water), this index may not be accurate. Third, differences in plant allocation may decouple total relative resource use from biomass gain. For example, early-season annuals allocate a greater proportion of their yearly growth to reproduction than do bunchgrasses (Armstrong 1991), which would lead to lower biomass gain in E's than if resources were allocated primarily to new leaves and stems.

Leaching losses and indirect plant effects

Despite differences in rooting depth, phenology, and total plant nitrogen among these functional groups (Gulmon et al. 1983, Chiariello 1989, Armstrong 1991, Hooper 1996, *in press*), we did not see decreased leaching losses with increasing diversity. This may result from three different mechanisms: climate, competition, and microbial immobilization.

Climate.—Seasonal complementarity in plant uptake could reduce leaching losses, but lack of water percolation in late spring and summer, when L's and P's have the greatest effect on inorganic N pools, prevents leaching losses at this time (Figs. 1, 6). Similarly, microbial immobilization over the summer could reduce losses at the start of the following rainy season by converting inorganic N to less labile organic forms in microbial biomass or soil organic matter (Vitousek and Matson 1984). However, slow mineralization outpaces both plant and microbial uptake, as seen by the increase in inorganic N pools from May to September (Fig. 1). The net result is that either plant uptake or microbial immobilization is necessary to prevent nitrogen losses

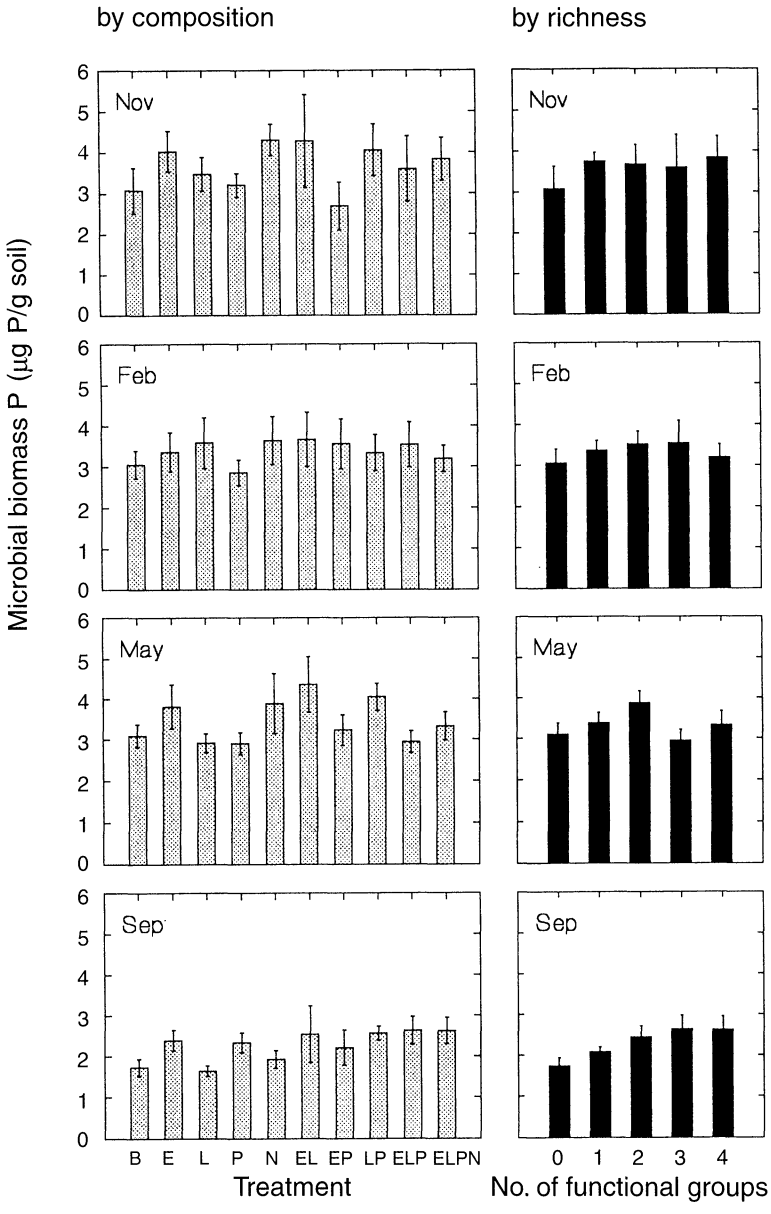

FIG. 8. Microbial biomass phosphorus (chloroform-labile P) plotted by functional group composition and richness (mean ± 1 SE, $n = 6$). See Table 7 for statistics.

by leaching or denitrification after the onset of the rainy season in the fall.

Competition.—Because annuals have not yet germinated in autumn when leaching begins, uptake by perennials in this season could be an important mechanism of retention for a pulse of nutrients when other species are not present (Marks and Bormann 1972, Jackson et al. 1988, Ewel et al. 1991, Lodge et al. 1994). However, we expect that autumn uptake of nitrogen by P's would be related to overall biomass of bunchgrasses, not just their presence or absence. Competition during previous seasons' growth, which reduced P biomass in mixture treatments (discussed above), can thus be important for the following season's uptake. Competition for a different resource (water) in the LP treatment may also influence nitrogen retention in the following season by reducing viable P biomass (Hooper, *in press*). If more than one resource limits plant growth, losses of any given nutrient (e.g., nitrogen leaching) may not decline with increasing diversity if competition for the second resource reduces the biomass of potentially complementary species. Though total resource use (averaged across all resources) may increase with increasing plant richness (Fig. 5), optimized retention for any single one (e.g., nitrogen) may not. Inclusion of even more functional groups in the

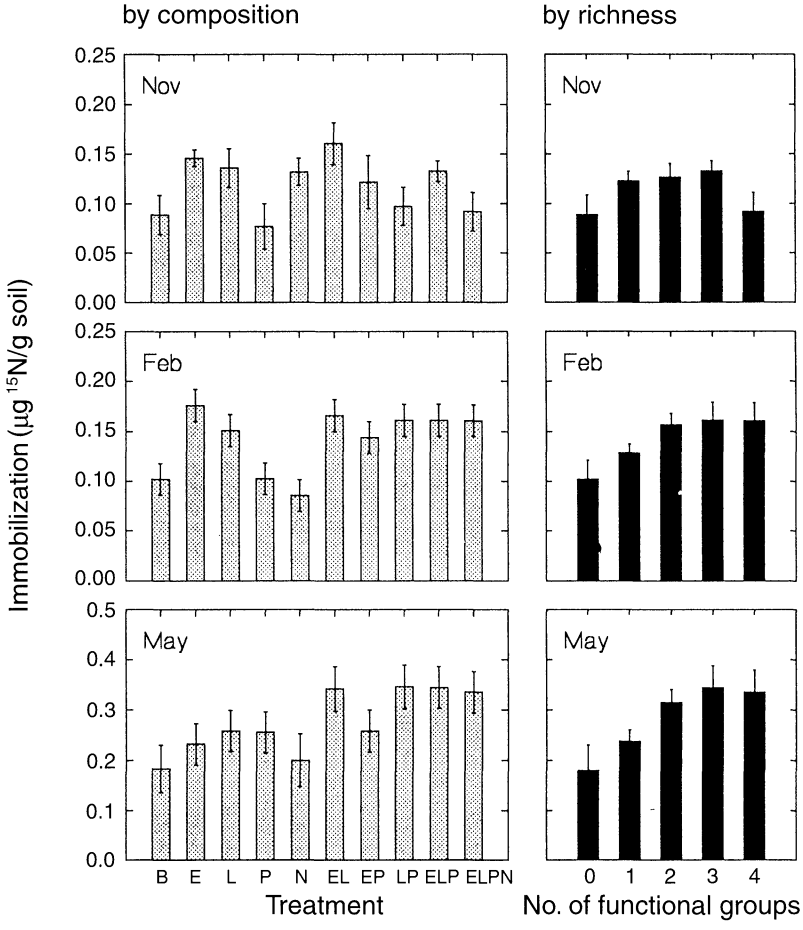

FIG. 9. Microbial immobilization (flush ^{15}N) plotted by functional group composition and richness. Values are adjusted means ± 1 SE (n = 6) from ANCOVA using TN as a covariate. Note the differences of scale among months; May data are not directly comparable to November and February due to a longer incubation time before fumigation in May. See Table 8 for statistics.

experimental communities could lead to greater use of the autumn pulse of nitrogen, if biotic or abiotic constraints at other times of the year do not limit their effectiveness.

Microbial immobilization.—Greater immobilization in annual-dominated treatments appears to equal autumn uptake by bunchgrasses (Fig. 9; Hooper 1996). In November, when bunchgrasses presumably would be important for complementary N uptake, they actually have a negative effect on MBN and immobilization (Figs. 7 and 9). Such effects on immobilization cannot be due solely to plant competition with microbes for N (Jackson et al. 1989, Schimel et al. 1989) because they take place in laboratory incubations in the absence of root uptake. Furthermore, microbes are generally considered better competitors for inorganic nutrients in short term experiments (Jackson et al. 1989, Schimel et al. 1989, Zak et al. 1990) and plants are thought to get the microbes' nutrient leftovers, rather than the other way around. Thus, indirect effects of plants on microbial immobilization, potentially via litter, appear to have as great an influence on ecosystem nutrient retention as does direct plant uptake (Vitousek and Matson 1984, 1985, Stark and Hart 1997).

Whether litter quality or litter quantity leads to these plant effects on microbial immobilization is not clear. On the one hand, more recalcitrant litter may lead to greater microbial N demand and higher immobilization (Aber and Melillo 1982, Melillo et al. 1982). The higher lignin : nitrogen ratios in early-season annual shoots and late-season annual stalks (Table 10; C. Benton, *unpublished data*) could lead to higher immobilization in annual-dominated plots. However, perennial bunchgrasses have significantly higher root biomass and significantly lower root %N than do early-season annuals (Hooper 1996, *in press*), suggesting greater potential for immobilization in P's (Wedin and Tilman 1990). On the other hand, greater quantities of labile litter could lead to higher microbial growth and higher immobilization. Bunchgrass roots are heavily suberized, long lived (Ares and Singh 1974, Clark 1977, Armstrong 1991), and immobilize little nitrogen relative to

TABLE 8. AN(C)OVA results for microbial ^{15}N immobilization (^{15}N flush; Fig. 9): (A) by composition; (B) by richness. Labels and statistics are as in Table 1.

Analysis	Nov	Feb‡	May‡
A) By composition			
AN(C)OVA			
r^2	0.436	0.596	0.485
Main effects and interactions			
E	+E > −E*	+E ≥ −E (0.008)	···
L	···	+L ≥ −L (0.013)	+L ≥ −L (0.007)
P	···	···	···
N	···	···	···
E × L	···	sat. (0.029)	···
E × P	···	···	···
L × P	···	···	···
E × L × P	neg. (0.038)	···	···
E × L × P × N	neg. (0.0193)	···	···
A priori comparisons			
Vegetation	···	+v ≥ −v (0.015)	···
Among SFG's			
E vs. L	···	···	···
E vs. P	E ≥ P (0.007)	E > P*	···
E vs. N	···	E > N**	···
L vs. P	L ≥ P (0.020)	L ≥ P (0.034)	···
L vs. N	···	L ≥ N (0.005)	···
P vs. N	N ≥ P (0.030)	···	···
Averaging	No significant differences		
B) By richness			
AN(C)OVA			
r^2	0.227	0.386	0.444
Number of FG's	···	*	*
Linear trend	···	**	**
Differences among levels of richness	No significant pairwise comparisons		
Regression			
r^2	0.136	0.355	0.416
Slope	···	0.016**	0.043**

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
‡ Pretreatment total soil nitrogen (TN) was used as a covariate.

aboveground litter in some experiments (Seastedt et al. 1992). Annuals may encourage microbial growth and immobilization by having less recalcitrant root litter and/or by having higher root turnover (Hart et al. 1993, Hooper 1996). These different hypotheses emphasize the need for more information about how the quantity and quality of root and shoot litter affect microbial growth and nitrogen demand at the ecosystem level.

Composition and diversity effects

Our experiments demonstrate that the phenologically-defined functional groups differed in their effects on ecosystem nutrient cycling. Productivity, N pools, and the distribution of these above and below ground, varied among groups (Gulmon et al. 1983, Armstrong 1991, Hooper 1996, *in press*). Amount and quality of aboveground litter also varied, with a resulting influence on decomposition and N flow (C. Benton, *unpublished data*; Hooper 1996). These differences, as well as phenology, led to differences in the patterns of resource use and availability across seasons, particularly for nitrogen and water. They also led to differences in the importance of plant and microbial mechanisms of ecosystem N retention, and in leaching losses among the single functional group treatments. We chose functional classifications based primarily on phenology because that is a well-studied aspect of the serpentine grassland system in California, but also because functional groups based on timing and spatial distribution of resource acquisition could logically affect ecosystem processes related to resource use and retention. Functional groupings may differ for different resources, different processes, or different ecosystems.

That ecosystem processes diverge in response to the different functional groups in single group treatments is a separate issue from how these groups and their traits combine in more diverse systems. We expect complementarity to be greater when species differ strongly in phenological or morphological traits, leading to an increasing response of productivity or nutrient retention with increasing species richness. Still, complementary behavior explains only a part of how these traits combined in mixture treatments. In particular, dominance by E's and L's had a large influence on

TABLE 9. ANCOVA results for nitrification potential (Fig. 10): (A) by composition, (B) by richness. Labels and statistics are as in Table 1. Pretreatment total soil nitrogen was used as a covariate in all months.

Analysis	Nov	Feb	May
A) By composition			
ANCOVA			
r^2	0.556	0.542	0.560
Main effects and interactions			
E	+E ≤ −E (0.031)	...	...
L	...	...	...
P	...	...	...
N	...	...	...
E × L	...	...	...
E × P	...	...	...
L × P	neg.†	...	...
E × L × P	...	...	...
E × L × P × N	...	...	...
A priori comparisons			
Vegetation	+v < −v†	...	...
Among SFG's	No significant differences		
Averaging	LP > avg.*	...	...
B) By richness			
ANCOVA			
r^2	0.389	0.509	0.491
Number of FG's	...	...	...
Linear trend	†	...	...
Differences among levels of richness	B > 1†	...	...
Regression			
r^2	0.302	0.446	0.480
Slope	...	...	...

Note: Data were natural-log transformed to homogenize variance or to improve normality before analysis.

inorganic N pools and soil moisture, respectively. These properties and others (e.g., microbial biomass, microbial immobilization) depended on certain functional groups or combinations of functional groups, so that ANOVA by composition explained much more of the variance across treatments than did analysis by level of richness alone. That is, knowing *who* the component functional groups were, rather than just *how many* there were, explained much more about the nutrient cycling properties of the different experimental treatments. Ewel et al. (1991) also reported effects of plant composition on nutrient dynamics that at least equalled effects of diversity (Ewel et al. 1991). Losses of cations under trees grown in monoculture compared to losses under previous monoculture maize rotations declined to levels equivalent to much more diverse successional systems (>100 species). This presumably resulted from the tree's perennial root structure and higher organic matter inputs (Ewel et al. 1991). Therefore, while there may be broad trends of response of ecosystem properties to plant richness (e.g., saturating curves; Swift and Anderson 1993, Vitousek and Hooper 1993, Tilman and Downing 1994, Field 1995, Naeem et al. 1995, Tilman 1996, Tilman et al. 1996), an idiosyncratic pattern (Lawton 1994, Naeem et al. 1995) may be the most likely when looking at the response of a given process in a given system as functional groups are gained or lost. The response will depend on the properties of the species in question, of the other species in the system, and the interactions between them (Lawton and Brown 1993, Chapin et al. 1995, 1996).

Dominance by E's for inorganic nitrogen in winter and by L's for soil moisture in summer led to significant statistical effects of functional group richness on these resource pools. Looking at patterns of ecosystem response only from the restricted view of the number of species or functional groups, however, obscured the fact that mechanisms were due to differences in composition, not necessarily to species richness per se and resource use complementarity. Controlling for composition should therefore be a fundamental part of experiments assessing diversity effects on ecosystem properties. Still, the effects of dominant species may be ecologically significant if increasing diversity on average increases the probability that such dominants will occur in a community and utilize resources that could otherwise be lost (e.g., to leaching). This is analogous to Tilman and Downing's (1994) proposed mechanism for drought resistance in more diverse communities. Dominant species or functional groups may not be those with greatest biomass in monoculture (Naeem

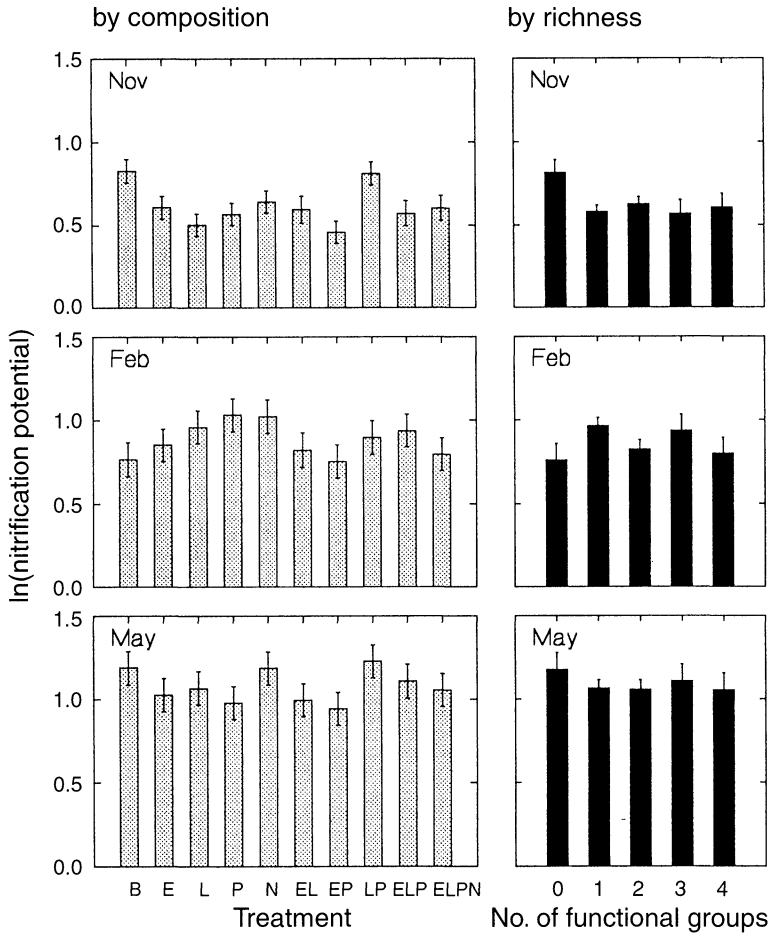

FIG. 10. Nitrification potential plotted by functional group composition and richness. Values are adjusted means and standard errors (n = 6) from ANCOVA of natural-log transformed data, using TN as a covariate. Transformation done as $y = \ln(1 + y_0)$. Original data (y_0) are in micrograms N per gram soil per day. See Table 9 for statistics.

et al. 1996, Hooper, *in press*); other functional traits that influence species' interactions in mixtures might be more useful for predicting the response of ecosystem resource use as richness changes (e.g., nutrient reduction in monoculture; Tilman 1988, Wedin and Tilman 1990, Tilman and Wedin 1991*b*). For other processes, one or a few keystone species may exert strong control at the ecosystem level, but predicting which species are keystone may be difficult (Bond 1993, Lawton and Brown 1993).

TABLE 10. Litter quality for roots and shoots of the different functional groups: lignin, cellulose, and phosphorus in aboveground litter and nitrogen in aboveground litter and live roots; early-season annuals (E; *Plantago erecta* only), late-season annuals (L; leaf litter and 1-yr old standing-dead flower stalks), and perennial bunchgrasses (P). Values are means ± 1 SE, n = 2 for lignin and cellulose, n = 4 for N and P. Shoot data are from C. Benton (*unpublished data,*); root %N is from Hooper (*in press*). Within rows, values with different superscript letters are significantly different at $P < 0.05$ (Tukey post hoc comparisons).

	Functional group			
	E	L (stalk)	L (leaf)	P
Lignin %	16.53 ± 0.37[a]	18.41 ± 1.11[a]	14.16 ± 0.10[a]	7.15 ± 1.29[b]
Cellulose %	31.87 ± 0.06[b]	42.45 ± 0.81[a]	29.31 ± 1.39[b]	29.18 ± 1.33[b]
%N	0.710 ± 0.091[b]	0.753 ± 0.051[ab]	0.948 ± 0.022[a]	0.426 ± 0.021[c]
%P	0.127 ± 0.017[a]	0.040 ± 0.004[b]	0.067 ± 0.003[b]	0.029 ± 0.001[b]
N/P	5.61 ± 0.12[c]	19.23 ± 0.45[a]	14.12 ± 0.33[b]	14.77 ± 1.02[b]
Lignin/N	26.9 ± 1.7[a]	24.5 ± 1.4[a]	15.2 ± 0.3[a]	17.6 ± 4.1[a]
Lignin/P	151 ± 16[b]	453 ± 10[a]	212 ± 4[b]	251 ± 52[b]
Root %N	1.36 ± 0.12[a]	1.15 ± 0.03[a]		0.75 ± 0.09[b]

This experiment differed from previous approaches (Naeem et al. 1994, 1995, 1996; Tilman et al. 1996) by using functional groups rather than species as the experimental unit. We anticipate that the mechanisms underlying the response of ecosystem nutrient use and/or retention to either functional group or species richness should be similar (e.g., complementarity, dominance, etc.). Despite the similarity of mechanisms, some of the patterns we observed may be related to the use of functional groups rather than species. By adding and removing entire groups of species, we prevented any compensatory response by other species within the same group. Therefore, we expect to see greater changes when eliminating whole groups instead of single species—until elimination of the last species of a group (Lawton and Brown 1993). Compensatory responses, in which some species increase growth or nutrient uptake in response to reduction in growth or uptake of a competing species, could be an important mechanism for stability of ecosystem processes. Greater stability could occur, for instance, if species within a functional group based on resource acquisition have different sensitivities to environmental conditions (Tilman and Downing 1994, Frost et al. 1995). Assessing ecosystem response to richness/diversity of species *within* functional groups (e.g., stability over different climate regimes) is an important component of understanding ecosystem processes, but was not attempted here.

Plant composition and richness effects interacted with abiotic conditions such that the relative effects of the functional groups, and in some cases the relative importance of composition and richness, differed with season. For microbial biomass N and P, inorganic N pools, available P pools, and leaching losses, differences among seasons were much greater than differences among treatments within a season. For example, though the size of the microbial biomass nitrogen and phosphorus pools decreased substantially in the dry season, positive effects of plant diversity (independent of plant composition) on microbial processes was most significant in May and September (Tables 2 and 4). Plant diversity may be more important for microbes in summer months because plant facilitative effects could outweigh negative effects on microbes in the drier, more stressed environment (Bertness and Callaway 1994). This raises the question of under what conditions we will most likely see ecosystem-level effects of diversity. On the one hand, under harsh environmental conditions, there may be more opportunity for facilitative interactions among species (Fowler 1986, Bertness and Callaway 1994, Berkowitz et al. 1995), similar to what we saw for diversity effects on microbial processes in the summer dry season. On the other hand, strong abiotic controls may then dominate process rates and restrict the expression of species-level functional traits at the ecosystem scale (Schimel et al. 1995, Vinton and Burke 1995). Knowledge about the relative roles of richness and composition will be important for understanding the behavior of intact ecosystems as species are gained or lost (Mooney and Drake 1986, Wilson 1992), as well as for improving yield and sustainability of low-diversity managed systems (Steiner 1982, Vandermeer 1988, Cannell et al. 1992, Swift and Anderson 1993). More mechanistic information on how functional attributes of species combine in diverse communities will help us better resolve how, when, and where differences in and diversity of these attributes are important for predicting ecosystem processes.

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APPENDIX

STATISTICS

Analysis of variance by composition

We analyzed the data for the full set of experimental treatments using analysis of variance in the General Linear Models command of SYSTAT (SYSTAT 1992). We used a factorial ANOVA of E, L, and P treatments with the addition of an N main effect and E × L × P × N interaction, as well as a categorical blocking factor (BLK = 1–6) to account for effects of location in one of the six replicate blocks. The ANOVA design essentially treats the E, L, P factorial as a separate analysis from N main effects and interactions. The latter includes only those treatments that are directly comparable: the contrast for N main effects is B + ELP − N − ELPN = 0; the contrast for the E × L × P × N interaction is B + ELPN − N − ELP = 0. We examined all residuals for homogeneity of variance and normality, using natural-logarithm transformations when necessary to improve normality or inequality of variance. When preliminary tests indicated significance, we included either total soil nitrogen (TN) or total soil phosphorus (TP) as a covariate (see *Methods: Total soil nitrogen and phosphorus*). We did not attempt to analyze the data by repeated measures or multivariable analysis of variance (MANOVA) because analysis of individual variables usually indicated different main effects and interactions for different variables in different seasons. These would have shown up as interactions (e.g., time × treatment) and necessitated the more detailed analyses anyway.

In addition to ANOVA main effects and interactions, we used the following a priori contrasts to analyze for effects of plants, functional groups, and diversity:

1) vegetation effect: bare treatment = all vegetated treatments,

$$9B = E + L + P + N + EL + EP + LP + ELP + ELPN;$$

2) single functional group (SFG) differences: pairwise comparisons of single functional group treatment means;

3) averaging: mixture treatments = average of component single functional group treatments.

Because opinions differ as to the appropriate approach for adjusting levels of significance for multiple a priori comparisons (Sokal and Rohlf 1981, Neter et al. 1990, Stewart-Oaten 1995), we corrected P values from these tests and the ANOVA table using Kimball's inequality (Neter et al. 1990) for the appropriate number of tests. This is the same as the "Dunn-Sidak" method, which Sokal and Rohlf (1981:261) recommend for use with nonorthogonal contrasts, and gives confidence limits very similar to, but slightly less restrictive than, Bonferroni corrections. Because these corrections are conservative, we refer to "statistical significance" as cases in which experimental P values fall below P values for familywide confidence limits of 90%. For example, with 21 contrasts for the full model with all treatments, familywide confidence levels of 90% are maintained when individual contrasts have $P < 0.005$. "Nonsignificant trends" refer to cases in which uncorrected P values fall below 0.05, but not below the Kimball corrected value.

Analysis of plant biomass or nitrogen yields does not allow evaluation of the full ANOVA model because values for the bare plot are meaningless (Hooper 1996, *in press*). We therefore used a combined means and effects model and only included cases from the vegetated plots. We determined main effects and partial two-way interactions by contrasting the weighted means of all plots containing a given functional group with all plots lacking it, excluding the bare plots. For example, a test for E main effects without bare plots is

$$3E + 3EL + 3EP + 3ELP + 3ELPN - 5L - 5P - 5LP = 0.$$

Determining interactions in this situation requires balancing of cell means such that a full test of interactions is not possible. When bare plots must be ignored, the test for a partial E × P interaction amounts to the contrast: EL + LP − L −

ELP = 0. This is equivalent to testing for an E × P interaction on a background of L's. Because neither the single functional group plots nor the strictly two-way combination of them (E, P, and EP in this example) appear in the test, some power to detect potential differences is lost. Neither can three- and four-way interactions be tested in this experimental design. The consolations are that (1) the averaging contrasts allow testing for biologically interesting "interactions" for the omitted combinations, and (2) there is better interpretation of the partial interaction tests because the results are not skewed by meaningless two-, three-, and four-way interactions due only to the bare plot.

Analysis of variance by richness

To test effects of level of functional group diversity (richness) on nutrient cycling parameters, we also performed one-way ANOVA and ANCOVA using number of functional groups (FG) as the treatment. We also included categorical block effects (BLK) and, where necessary, total soil nitrogen or phosphorus (TN, TP) as a covariate. Although there are unequal numbers of treatments in each level of diversity [0 = bare plots (n = 6); 1 = E, L, P, and N (n = 24); 2 = EL, EP, and LP (n = 18); 3 = ELP (n = 6); and 4 = ELPN (n = 6)], SYSTAT's regression coding of the ANOVA under General Linear Models handles the unbalanced design appropriately (Neter et al. 1990, SYSTAT 1992). However, due to resource constraints, only the one- and four-group treatments include nitrogen-fixers, which may bias any linear trends. These cases are discussed in the *Results* section when they arise. We performed post hoc pairwise comparisons of all cell means, corrected by Sheffé's method (necessary for unbalanced designs; Sokal and Rohlf 1981), and a test for a linear trend in cell means as diversity increases by using the contrast for first-order polynomials (SYSTAT 1992). We also did a similar analysis using a regression approach in which FG is taken as a numeric variable, BLK is still categorical, and TN or TP are noninteracting covariates (when necessary). This appears to give a more robust estimate of a monotonic trend than does the polynomial coding following ANOVA.