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Soil microorganisms of a native shrub and exotic grasses along a nitrogen deposition gradient in southern California

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Abstract

Both anthropogenic nitrogen deposition and exotic annual grass invasion are occurring in the coastal sage scrub vegetation (CSS) of southern California. A study was carried out to determine the effects of these changes on soil microbial communities. Soils were sampled under exotic grasses and the native shrub *Artemisia californica* along an urban-to-rural N deposition gradient, and in N-fertilized plots in a rural site with low deposition. Arbuscular mycorrhizal (AM) colonization of *A. californica* was highest in low-N soils, but annual grasses (mainly *Bromus madritensis*) were colonized primarily by a fine endophyte that showed no pattern of response to soil N level. In addition, annual grasses generally had higher colonization by nonmycorrhizal, primarily septate, fungi in high N soils, while nonmycorrhizal colonization of *A. californica* was low in all soils. Spore density declined in the rhizosphere of *A. californica* with elevated N, but not of *B. madritensis*. Fatty acid methyl esters (FAME) were extracted from soil and their profiles were used to describe the soil microbial communities. Principal components analysis of FAME profiles showed a significant but weak relationship with levels of soil N. At the low N-deposition site that received N fertilizer, host plant was more important than soil N in determining FAME profiles. The most abundant fatty acids were the biomarkers for AM fungi. The different microbial communities of the two species, especially the predominance of fine endophyte and nonmycorrhizal fungi in roots of *B. madritensis*, warrant further research on functional responses to understand how these microorganisms may be involved in the invasion of native shrubland by exotic grasses.

Keywords: Arbuscular mycorrhizal fungi; *Artemisia californica*; *Bromus madritensis*; Coastal sage scrub; Nitrogen; Fatty acids

1. Introduction

Anthropogenic nitrogen (N) deposition has been implicated in promoting invasions and species shifts of plant communities in Europe and the United States (Vitousek et al., 1997; Bobbink et al., 1998), and in altering soil microbial communities (Johnson et al., 1998; Bardgett et al., 1999b). However, the combined impacts of elevated N and invasive species on the soil microflora is little understood, as the soil microbial communities may be altered by the invasive plant species themselves (Belnap and Phillips, 2001; Ehrenfeld, 2003; Bever, 1994; Vinton and Burke, 1995; Bardgett et al., 1999b; Marschner et al., 2001). Several studies have specifically examined the effects of host plant on arbuscular mycorrhizal (AM) fungal spore species composition. For instance, adjacent plants of different species in a Minnesota, USA, grassland had different (AM) fungal spore communities (Johnson et al., 1992), and exotic grasslands in southern California had a different species composition of AM spores from native grasslands (Nelson and Allen, 1993; Gillespie and Allen, 2006).

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The impacts of elevated N have been assessed for various taxa of soil microorganisms. Some consequences of increasing soil N include increases or decreases in microbial biomass depending on taxa (Lovell et al., 1995; Johnson et al., 1998; Bardgett and McAlister, 1999), decreased fungal-to-bacterial ratios (Bardgett et al., 1999b), and decline of some microbial species (Arnebrant et al., 1990). Elevated N caused a decrease in arbuscular mycorrhizal (AM) root colonization in four of five sites, with the initial soil fertility (N:P ratio) controlling plant response to N (Johnson et al., 2003). Elevated N also caused a decline in AM spore density and richness in three sites (Johnson, 1993; Egerton-Warburton and Allen, 2000; Egerton-Warburton et al., 2001). The impacts of elevated N may be determined by plant and microbial taxa and initial soil conditions such as fertility or disturbance level, so a prediction of expected microbial changes under elevated N is difficult to make. Understanding the interaction of elevated soil N and host plant on soil microflora may help explain the dominance of invasive species; soil microbial changes may be a direct consequence of N deposition, but they may also be due to changes in composition of the plant community because plants provide the substrate for growth of saprotrophic and symbiotic soil organisms.

In southern California N deposition may be as high as 45 kg N ha^{-1} $year^{-1}$ (Fenn et al., 2003a,b). The coastal sage scrub (CSS) vegetation has been experiencing conversion from a semi-deciduous shrubland to Mediterranean annual grassland over the past 40 years, which is more pronounced in areas of high N deposition (Minnich and Dezzani, 1998; Allen et al., 1998; Fenn et al., 2003b). Species shifts in AM fungi have been observed in this region under elevated N, with a loss of large-spored genera such as *Scutellospora* and *Gigaspora*, and increased abundance of small-spored *Glomus* species along a gradient of high to low N deposition (Egerton-Warburton and Allen, 2000). Losses of mycorrhizal species diversity may have negative consequences for the plant community. For instance, soil N eutrophication in Central Europe resulted in reduced sporocarp density and reduced ectomycorrhizal diversity and colonization, and subsequent reduced plant growth (Wallenda and Kottke, 1998; Wöllecke et al., 1999). Changes in composition of AM mycorrhizal fungal populations under elevated N in various locations in North America also resulted in reduced growth of the host plants (Johnson, 1993; Corkidi et al., 2002). Diversity of mycorrhizal fungi contribute to the maintenance of plant diversity and ecosystem functioning (van der Heijden et al., 1998; Allen et al., 2003), so understanding shifts in microbial communities under N deposition or plant invasions may help to explain aboveground community responses.

Various techniques may be used to study changes in soil microorganisms. For AM fungi, identifying and counting spores has been used to determine shifts in species composition, although the relationship of spores produced in the rhizosphere with internal colonization is not precise in species mixtures. However, studies using spore counts to assess differences in AM species composition have recently been confirmed for internal infection using molecular techniques (Vandenkoornhuyse et al., 2002), and show that spore counts are a useful technique. Furthermore, roots may harbor many fungal species in addition to AM fungi (Vandenkoornhuyse et al., 2003), and these fungi may also be observed microscopically (Rillig et al., 1998). An indirect technique to detect microbial change is to use fatty acid methyl ester profiles (FAME) to determine differences in a broader range of fungal and bacterial taxa (Cavigelli et al., 1995; Zelles, 1999; Buyer et al., 1999; Ritchie et al., 2000). Certain fatty acids can be used as biomarkers to assess the composition of the microbial communities that are represented by fatty acid profiles (Bentivenga and Morton, 1996; Stahl and Klug, 1996; Olsson, 1999).

The objectives of this study were to evaluate colonization by mycorrhizal and nonmycorrhizal fungi, as well as changes in microbial FAME profiles, in an abundant invasive annual grass, *Bromus madritensis*, and a dominant native shrub, *Artemisia californica*, in CSS vegetation invaded by Mediterranean annual grasses in southern California. Both species were sampled along a documented urban-to-rural N deposition gradient with increasing levels of soil N near the urban areas (Padgett et al., 1999). To control for the site-to-site variability inherent in any natural gradient, samples were also collected in N-fertilized plots at the rural end of the gradient in a region of low N deposition.

2. Materials and methods

2.1. Site description

The N deposition gradient occurs in the Riverside-Perris Plain, western Riverside County, in CSS vegetation ranging from northern urbanized sites to southern rural sites (Table 1; a site map is published in Padgett et al., 1999). The gradient is located in a Mediterranean-type ecosystem where the rainy season occurs mainly from November to April. The 50-year average annual precipitation is 280 mm in Riverside.

Table 1
Geographical location and elevation of the sample sites along the N-deposition gradient

Site	Abbreviation	Latitude	Longitude	Elevation (m)
Jurupa Hills	JH	34°03′	117°36′	350
Mockingbird Reservoir	MR	33°54′	117°20′	340
Waterman Road	WR	34°11′	117°20′	800
Botanic Garden	BG	33°58′	117°17′	350
Pedley	PE	34°01′	117°37′	340
Motte Reserve	MO	33°48′	117°15′	550
Lake Mathews	LM	33°51′	116°56′	467
Hemet	HE	33°43′	117°10′	600
Lake Skinner	LS	33°37′	117°20′	340
Santa Margarita Reserve	SM	33°29′	117°09′	433

All sites were located on similar soil (sandy to gravelly loams on decomposed granites of the Cienega and Fallbrook series, United States Department of Agriculture, 1971). Bicarbonate extractable P along the gradient was moderate to high at all sites with 13–46 μg P/g soil, exchangeable K was 159–447 μg/g and soil organic matter was 1.4–3.7% (Padgett et al., 1999). Sites were selected with similar vegetation consisting of stands dominated by *A. californica* Less. with an understory dominated by *B. madritensis* ssp *rubens* (L.) Husnot., and several other genera of exotic annual grasses (species of *Avena*, *Hordeum*, and *Vulpia*). Although the native shrub cover has declined up to 90% in the urban areas (Minnich and Dezzani, 1998), sites were chosen to include both native shrubs and exotic grasses at all sample locations. *A. californica* is a summer deciduous shrub that is among the dominant species in CSS vegetation (DeSimone and Burk, 1992), and *B. madritensis* spp. *rubens* has become one of the most abundant exotics. The N deposition gradient is about 90 km long ranging north to south from urban to rural western Riverside County (Padgett et al., 1999). The northernmost site, Waterman Road (WR), is located adjacent to the higher elevation chaparral vegetation. The northern end of the gradient has received N deposition for more than 40 years (Egerton-Warburton et al., 2001), primarily as nitrogen oxides that originate from automobile emissions, while the rural southern end has relatively clean air. Atmospheric concentrations of NO_3^--N are high with values up to 32 ppb in the urban northern end of the gradient, and low values of 8 ppb in the southern rural end (Padgett et al., 1999).

To control for the inherent variability that occurs along any natural gradient, we also established N-fertilizer plots near the southern end of the gradient at the Lake Skinner (LS) site. Since 1994, 10 replicate 5 m × 5 m plots have been fertilized annually with 60 kg N ha^{-1} $year^{-1}$ (F treatment) and another 10 were maintained as unfertilized controls (NF) (Allen et al., 1998; Allen, 2004). This value is double the annual amount of N that is deposited on shrublands at the northern end of the gradient (Bytnerowicz et al., 1987).

2.2. Sample collection

Soil samples were collected from under one dominant native shrub species and from the rhizosphere of the exotic grasses. Five replicate soil samples were collected under the canopy of *A. californica* and in shrub interspaces dominated by *B. madritensis*. Sampling locations were selected randomly within a 1 ha area in each site. Samples were collected with a 2.5 cm diameter corer to a depth of 5 cm. For each sample, three adjacent core samples were taken under the *A. californica* canopy or in the grass-dominated interspace. The three cores were combined to assure sufficient soil for all the analyses. Roots of *A. californica* and grasses were identified by morphology and color and picked from samples for staining. The soil was sieved through a 2-mm sieve for other analyses.

2.3. Soil nitrogen

Soil samples for N determination were collected in August of 1995–1998 along the gradient and from the experimental plots. Five samples were collected under *A. californica* and five under annual grasses. Dry season sampling was conducted because inorganic N accumulates at this time of the year, while wet season values are low even in high deposition sites (Padgett et al., 1999). Ammonium and nitrate were analyzed by KCl extraction and N detected by the diffusion conductivity method (Carlson, 1978) at the Analytical Laboratory, University of California, Davis.

2.4. Mycorrhizal fungal colonization

Root sampling for mycorrhizal fungal colonization was performed in 1997 at seven sites: Botanic Garden (BG), Jurupa Hills (JH), Mockingbird Reservoir (MR), Pedley (PE), Motte Reserve (MO), Lake Skinner (LS), and the Santa Margarita Ecological Reserve (SM). Five replicate root samples were cored as described above from beneath shrubs and interspaces dominated by grasses at each site in February, when root colonization by AM fungi was highest (Sigüenza, 2000). Samples from the fertilized and control plots at Lake Skinner were also analyzed using the same techniques, but increasing the replication to 15 cores from each shrub and grass samples from fertilized and control plants (60 total). The most abundant grass at each site was *B. madritensis*, but roots of other annual grass species, especially *B. diandrus* may have been included. Because grasses and other species occurred under the shrubs, roots of *A. californica* were separated from grasses based on morphology (shrub roots were darker and coarser). To quantify the mycorrhizal colonization, the washed roots were stained with trypan blue (Koske and Gemma, 1989), mounted on slides and evaluated by the intersection method at 100× magnification (McGonigle et al., 1990). Total, arbuscular, vesicular, coil, and nonmycorrhizal colonization were scored in 50 random observations per core. Preliminary analyses showed this sample size was adequate, as additional observations per core did not significantly change percent colonization values. In addition, fine versus coarse endophytes were scored based on morphology. Coarse endophytes had intraradical hyphae that ranged from 2 to 10 μm in diameter, with vesicles produced at the hyphal apex. Fine endophytes had hyphae thinner than 2 μm, often with intercalary vesicles, thin, lightly stained walls and often required observation at 400× to assure identification (Thippayarugs et al., 1999). Finally, nonmycorrhizal fungi were also assessed for presence/absence in each of the 50 observations per sample. They were primarily septate hyphae between cortical cells that may belong to a number of unidentified fungal species.

2.5. Spore counts

For mycorrhizal spores, five replicate soil cores each from shrub and grass were collected from each site in September 1997 when the highest spore density occurs (Egerton-Warburton and Allen, 2000). Spore samples were collected from all ten sites listed in Table 1, as well as the fertilized plots at Lake Skinner. Spores were extracted by the sucrose centrifugation method (Allen et al., 1979) in 5 g fresh weight soil samples and counted under the dissection microscope at 50×. Spore density was corrected for soil moisture using dry mass of soil subsamples dried at 65 °C to constant mass. Spores from the fertilized and control plots at Lake Skinner were also collected in 1998.

2.6. FAME (fatty acid methyl ester) profiles

For fatty acid methyl ester extraction, sampling was performed in 1998 and 1999 along the gradient. In 1998 the fertilized plots were also included. Soil sampling was done as for soil N and mycorrhizal spores, but soil samples were frozen immediately after collection. Samples were freeze-dried for 48 h, passed through a 2-mm sieve and stored frozen. Fatty acids were extracted following a protocol based on the MIDI system (Microbial ID, Inc., Newark, DE, USA).

Fatty acid analysis was performed on all samples using a slight modification of the Buyer et al. (1999) procedure, which is based on the procedure by Cavigelli et al. (1995). A Hewlett-Packard (Wilmington, Delaware) 5980 gas chromatograph with flame ionization was used for detection. Fatty acids were identified by retention time according to the MIDI eukaryotic method (Microbial ID, Inc., Newark, Delaware).

2.7. Statistical analyses

All data were tested for normality and homoscedasticity, and square root or log transformed as needed. Percent colonization data were arcsine transformed prior to statistical analyses.

Soil extractable nitrate and ammonium data were subject to one-way ANOVA to compare the different sites. In addition, regressions were run using latitude along the north–south urban to rural gradient as the independent variable, and soil nitrate or ammonium as the dependent variable.

The variables measured for the mycorrhizal colonization and spore data were subject to ANOVA. The values from the N deposition gradient were each subject to one-way ANOVA, and the values from the Lake Skinner N-fertilizer experiment were subject to two-way factorial ANOVA with two levels of N and two host plant species. The three most abundant fatty acids from the FAME analyses were subject to ANOVA to compare their relative abundance in sites along the N-deposition gradient and in the N-fertilizer plots at Lake Skinner. All ANOVAs were followed by $L.S.D._{0.05}$ to show significant differences among treatments or sites.

The mycorrhizal colonization and FAME data were further subject to principle components analysis (PCA) using PCOrd (MjM Software design, Gleneden Beach, Oregon). Not all response variables showed a uniform response to N-deposition site or N-fertilizer treatment, so the PCA was used to show differences among the N-fertilizer treatments or sites along the gradient. The PCs were calculated by weighted averaging using a correlation matrix. Number of significant PCs was determined by the broken stick method (Jackson, 1993). Regression analyses were done to show the relationships between soil N and the significant PCs for the mycorrhizal and FAME variables along the N deposition gradient. For the N-fertilized plots, two-way ANOVAs were run on the significant PCs from the N fertilizer and host plant treatments.

3. Results

3.1. Soil N

Soil analysis showed that dry-season levels of ammonium were similar along the gradient, but nitrate levels increased generally with distance from rural to urban sites (Fig. 1). The means of 4 years, 10 samples per site are shown. The sites are generally arranged from north to south (Fig. 1, Table 1). However, local differences in soils and/or wind flows caused some differences in rank, with Pedley having lower than expected soil N than its location near the most polluted site, Jurupa Hills, would suggest. A regression of latitude versus soil nitrate had $R^2 = 0.105$ and $p < 0.0001$, showing a significant increase in soil nitrate in the northern urban areas. The regression for ammonium was not significant ($p = 0.53$). We also checked for correlations of soil N with other edaphic factors, including soil extractable P and K, % total N, and organic matter, and while these varied from site to site they were not significantly related to nitrate, ammonium, or latitude. Thus the gradient can be described as a nitrate gradient, but is also described here for convenience as a set of four sites with high N, three sites with intermediate N, and three sites with low N. The elevated N of the fertilized plots at Lake Skinner is also shown (Fig. 1), and was not entered into the regression but was analyzed with the overall data using ANOVA. The extractable soil N values for the fertilized plots were as high as those of the high N deposition sites.

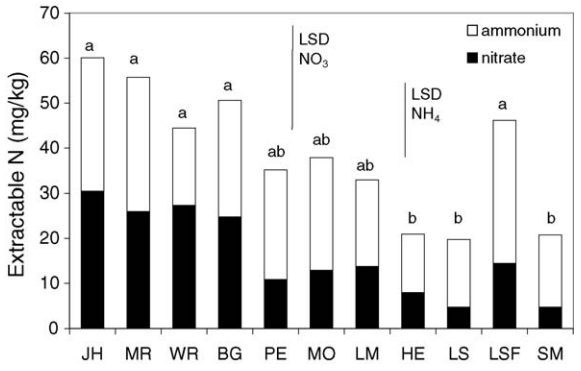

Fig. 1. Total extractable soil N along an anthropogenic N deposition gradient. JH: Jurupa Hills, MR: Mockingbird Reservoir, WR: Waterman Road, BG: Botanic Garden, PE: Pedley, MO: Motte Reserve, LM: Lake Mathews, HE: Hemet, LS: Lake Skinner, LSF: Lake Skinner fertilized with N, SM: Santa Margarita (see Table 1 for site locations). Bars are $L.S.D._{0.05}$ for ammonium and nitrate. Letters show significantly different means for the sum of nitrate plus ammonium.

3.2. Root fungal colonization and mycorrhizal spores

Mycorrhizal colonization (total, arbuscules, coils, and vesicles) of *A. californica* was highest at the low deposition Lake Skinner site, with Santa Margarita generally intermediate in value between Lake Skinner and the high and medium N-deposition sites (Fig. 2). The sum of individual AM structures (arbuscules, coils, vesicles) may be greater than total colonization because two or more structures often occurred in one microscopic observation. Because the fine endophyte was so low in *A. californica*, the total colonization value reflects primarily coarse endophyte. Conversely, annual grasses had much higher colonization by the AM fine endophyte than *A. californica*, sometimes with 50% of the total colonization attributed to fine endophyte. Fine endophyte colonization showed no pattern of response according to high or low N sites (Fig. 2). Although the impacts of elevated soil N were variable, as expected, along the N deposition gradient, the negative impacts of elevated soil N on mycorrhizal colonization of *A. californica* were confirmed in the fertilized plots (Fig. 3). Total, arbuscular, and vesicular mycorrhizal colonization were reduced by fertilization in *A. californica* but not in grasses. Conversely, fine endophyte colonization was not affected by fertilization (Fig. 3).

The Lake Skinner site had the highest AM spore density for both host plant species, but other patterns of spore density with soil N were not apparent along the gradient (Fig. 2). However, in the N-fertilized site, spore density was significantly higher under unfertilized than fertilized *A. californica* ($p < 0.001$). However, there was no effect of fertilization on spore density in annual grasses (Fig. 3).

Nonmycorrhizal fungal colonization (fungi other than mycorrhizal) was significantly greater in grasses in

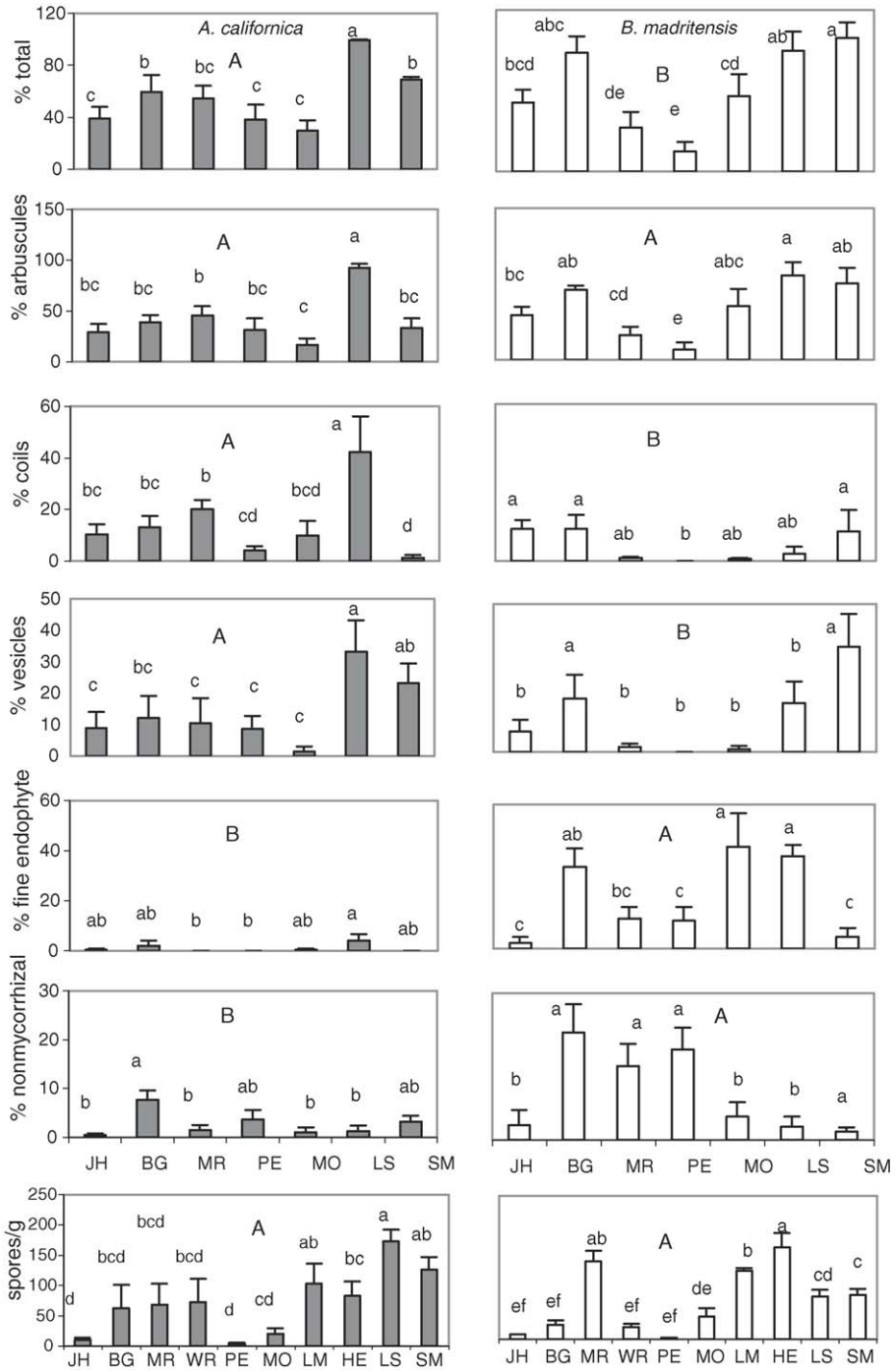

Fig. 2. Mycorrhizal colonization (total, arbuscular, coil, vesicular, fine endophyte), nonmycorrhizal colonization and spore density in *Artemisia californica* and annual grasses. The sum of individual AM structures (arbuscules, vesicles, coils, fine endophyte) may be greater than total colonization because two or more structures were often observed in one microscopic observation. Site abbreviations as in Fig. 1. Colonization data are from seven sites, spore data from 10 sites. Different capital letters show significant differences between species, lower case letters show significant differences between sites within species based on $L.S.D._{0.05}$.

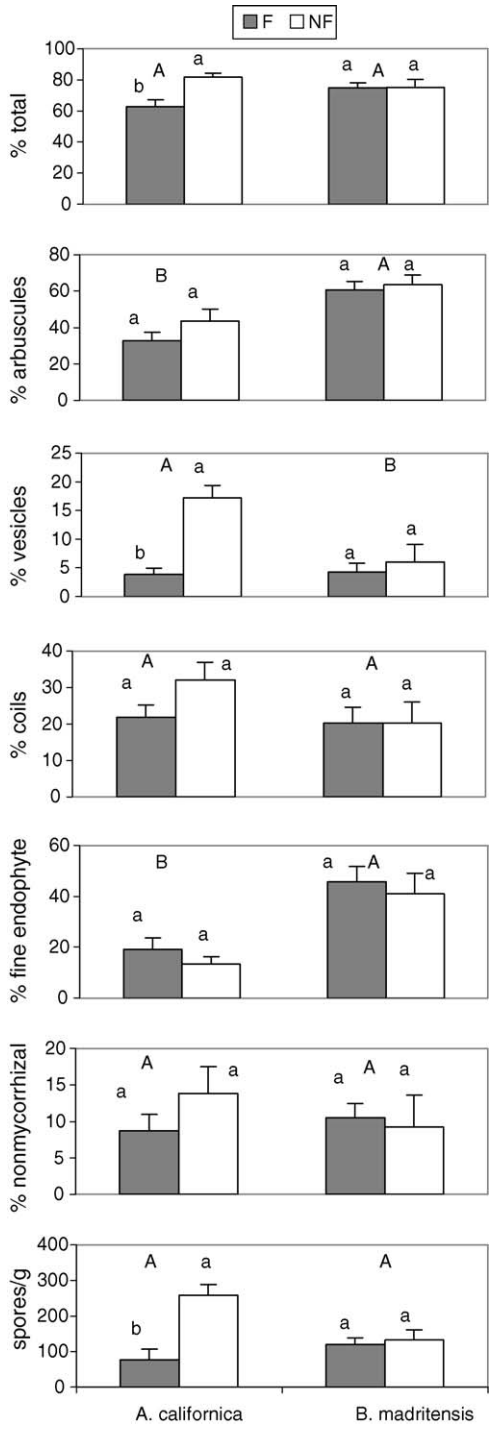

Fig. 3. Mycorrhizal colonization (total, arbuscular, coil, vesicular, fine endophyte), nonmycorrhizal colonization and spore density in the fertilized plots at the Lake Skinner low-N deposition site. F: fertilized with N, NF: nonfertilized. Different capital letters show significant differences between species, different lower case letters show significant differences between N treatments within species based on $L.S.D._{0.05}$.

the Botanic Garden, Pedley and Mockingbird Reservoir (high to moderate N sites) than in other sites (Fig. 2). At the Lake Skinner low-N site, colonization by nonmycorrhizal fungi showed no significant fertilization effect in either plant species (Fig. 3). The % nonmycorrhizal fungi was very low at the Lake Skinner site, and was not significantly different between shrub and grass.

In addition to the ANOVA's of Fig. 2, regression analyses were done on each of the fungal colonization variables versus soil nitrogen. These revealed several significant p (<0.05) showing the relationship between declining spore counts and increasing soil nitrate for *A. californica*, and declining total and arbuscular AM colonization with increasing nitrate for *B. madritensis*. However, R^2 values associated with these significant p-values were quite low, in the range of 0.2, and thus nitrate explained only a small portion of the variability of each of these individual fungal colonization measurements.

3.3. PCA of fungal colonization and spore density

Although the ANOVAs and regressions showed some trends in fungal colonization and spore characteristics,

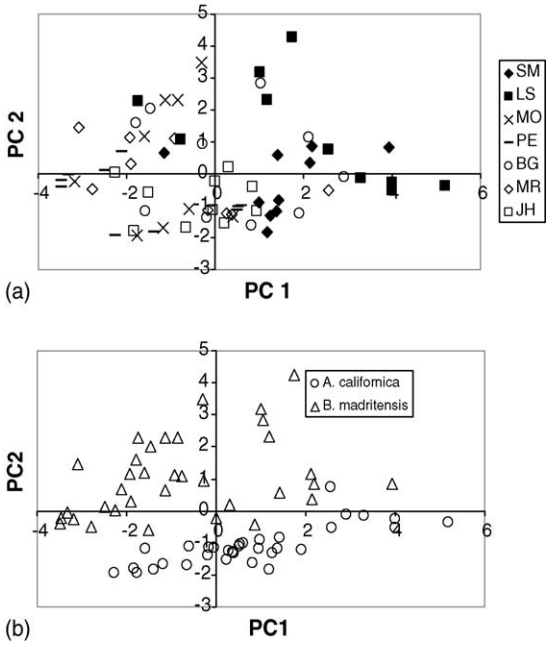

Fig. 4. (a) Principle components analysis (PCA) of fungal colonization and spore density variables (from Fig. 2) by sites along the N deposition gradient showing PC axes 1 and 2. (b) PCA of fungal colonization variables by host plants species. The first two PCs were significant according to the broken stick eigenvalue, and the scores for PC2 were significantly related to nitrate (Table 2A). Site abbreviations as in Table 1.

the inherent variability associated with the gradient made it difficult to define clear trends associated with N deposition. PCA was used to evaluate the relationships among the sites along the N gradient using the colonization variables (arbuscular, vesicular, coil, coarse and fine endophyte, nonmycorrhizal colonization and spore density). The low N sites (Lake Skinner and Santa Margarita) group together in a plot of the first two axes of the PCA, and the medium to high N sites form a more dispersed cluster (Fig. 4a). The first two PC axes of the colonization analysis were significant using the broken stick eigenvalue, and explained 60% of the variance (Table 2). The regression of soil N versus the PC scores for the first two PCs was significant for nitrate on PC2, but the regression for nitrate was not significant for PC 1 or for ammonium on either PC (Table 2). Grass and shrub colonization data points are shown separately in Fig. 4b, and *t*-tests of the PC scores for host plant were significantly different at $p < 0.001$ for both PCs, indicating the overall different fungal colonization responses of the two plant species.

The PCA of the mycorrhizal colonization and spore variables from the Lake Skinner N-fertilizer plots also showed significant effects of soil N level and host plant

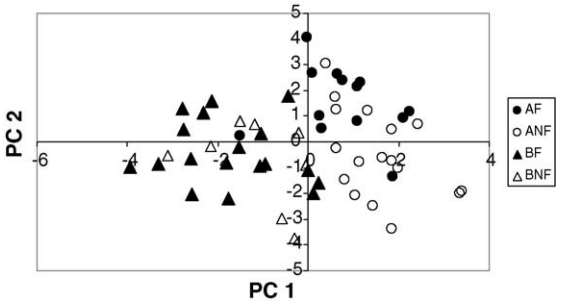

Fig. 5. PCA of fungal colonization variables (from Fig. 3) by host plant species and N fertilizer level at the N-fertilized plots showing PC1 and PC2. The first two PCs were significant according to the broken stick eigenvalue, and both were significantly related to host species and soil N concentration (Table 2C). A: *A. californica*, B: *Bromus madritensis*, F: fertilized, NF: nonfertilized.

(Fig. 5). The first two PCs were significant for this data set, with 63.8% of the total variance (Table 2). The two-way ANOVA showed a significant effect of both N fertilizer level and host plant on PC1 and PC2 scores. This indicates that, although individual colonization variables responded differently to soil N and host plant (Figs. 2 and 3), there was an overall combined response

Table 2
Cumulative % variance of the significant PC axes, and results of regressions and two-way ANOVAs

PC axis	Cumulative % variance	Regression			
		Nitrate		Ammonium	
		R^2	p	R^2	p
A. Fungal colonization on N gradient					
1	39.2	0.035	0.124	0.014	0.337
2	60.0	0.122	**0.004**	0.031	0.154
B. FAME on N gradient					
1	28.3	0.026	0.162	0.001	0.742
2	42.1	0.022	0.200	0.001	0.796
3	54.0	0.069	**0.020**	0.000	0.992
4	62.7	0.015	0.293	0.003	0.659

PC axis	Cumulative % variance	ANOVA		
		Species p	N fertilizer p	Spp × N p
C. Fungal colonization in N-fertilized plots				
1	33.4	**<0.001**	**0.037**	0.708
2	63.8	**0.004**	**0.002**	**0.025**
D. FAME in N-fertilized plots				
1	25.4	**0.002**	0.375	0.154
2	36.3	**0.042**	0.831	0.689
3	45.5	**<0.001**	0.269	0.852
4	53.9	**0.045**	0.693	0.935

Regressions show R^2 and p-values for the relationship between soil nitrate and ammonium on PC scores for (A) fungal colonization plus spores and (B) FAME profiles on the N deposition gradient. ANOVAs show effects of host plant species (exotic grass or native shrub) and N fertilizer treatment (or control) on PC scores for (C) fungal colonization and (D) FAME profiles at the Lake Skinner N-fertilized plots. Significant p-values are shown in bold.

by these variables that was consistent according to treatment and could be detected in the PC scores.

3.4. FAME profiles

Fatty acid methyl ester (FAME) profiles of the soils collected along the N gradient revealed shifts in the microbial community composition due to elevated soil N. A total of 22 fatty acids were extracted from the gradient samples in 1998. In all sites except Waterman Road the most abundant fatty acid was 16:00, and the next two most abundant were 16:1ω5C and 18:1ω9c, except at Mockingbird Reservoir (high N) where the former was more abundant than the latter (Table 3). At Waterman Road the composition and the proportion of fatty acids were different from the rest of the sites. The most abundant fatty acid at Waterman Road was in fact 19:0 10 methyl, which is not listed in Table 3 because it was unique to this site. The analysis of variance of the fatty acids showed that some high N sites, such as Jurupa Hills and Waterman Road had lower levels of the most abundant fatty acids compared to Lake Skinner and Hemet, two sites with lower N deposition (Table 3). However, there was considerable variability in abundance of fatty acids along the gradient, and their ability to differentiate among the sites is not evident among the three most abundant shown (Table 3). Except for one site (Motte Reserve) there were no significant differences between *A. californica* and annual grasses in FAME profiles (data not shown).

Table 3
Percent (S.E.) of the three most abundant fatty acids in the gradient sites and in fertilized plots at Lake Skinner in 1998

Gradient site	Fatty acids		
	16:1ω5c	16:00	18:1ω9c
Jurupa Hills	6.2 (1.2) c	20.2 (1.3) b	12.8 (0.5) b
Mockingbird Reservoir	18.0 (1.5) a	20.9 (0.4) b	10.2 (0.4) b
Waterman Road	1.9 (1.0) d	9.9 (2.6) c	1.7 (1.1) c
Botanic Garden	5.4 (1.3) cd	20.6 (0.5) b	8.7 (0.6) b
Pedley	4.2 (2.1) cd	26.9 (3.5) a	14.4 (3.7) ab
Motte Reserve	7.5 (1.4) bc	21.1 (1.8) b	20.3 (5.7) a
Lake Mathews	17.3 (1.8) a	22.6 (0.7) b	14.5 (2.2) ab
Hemet	7.1 (0.9) bc	23.8 (0.7) ab	19.5 (1.2) a
Lake Skinner (NF)	10.6 (1.1) b	24.9 (0.9) ab	12.8 (1.2) b
Santa Margarita	8.9 (0.8) b	26.5 (0.7) ab	8.9 (0.5) b
Fertilized plots			
AF	10.8 (1.1) bc	21.9 (1.5) a	11.7 (1.0) a
ANF	13.9 (1.7) ab	21.6 (1.2) a	13.0 (1.1) a
GF	7.9 (0.8) c	19.8 (0.5) a	10.8 (0.4) a
GNF	14.3 (0.8) a	20.9 (0.5) a	11.6 (0.6) a

F: fertilized with nitrogen, NF: not fertilized, A: samples from *Artemisia* rhizosphere, G: grass rhizosphere. Different letters indicate significantly different means using $L.S.D._{0.05}$.

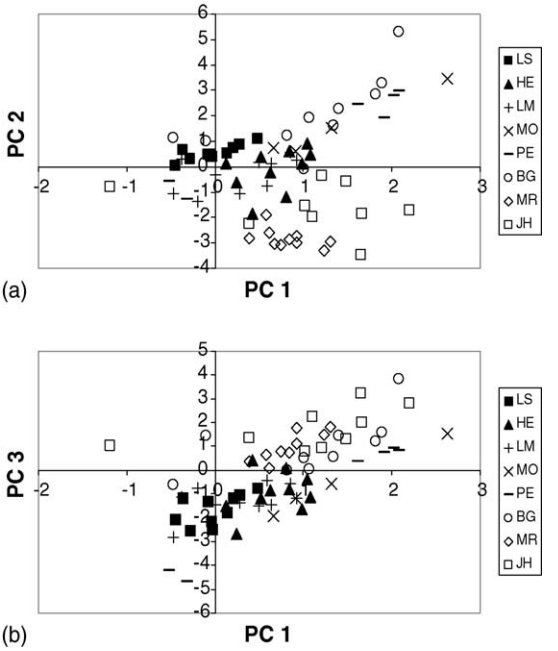

Fig. 6. PCA of soil fatty acids from the N deposition gradient for (a) PC1 vs. PC2 and (b) PC1 vs. PC3. The first four PCs were significant according to the broken stick eigenvalue (Table 2B), but nitrate was related to fatty acids only on scores for PC3.

The PCA for FAME in the N gradient sites included those fatty acids that were present in at least three of the sites, and revealed additional patterns of response to N (Fig. 6). Twenty-four fatty acids where used in the PC for the gradient, and the total number extracted was 28.

The first four PCs were included according to the broken stick eigenvalue method, and accounted for 62.7% of the variation (Table 2). The regression relationship between soil nitrate and the PC scores was weak, with a significant *p*-value only for PC3 (Table 2). The apparent separation of the sites is not explained by soil N except on PC3, indicating other factors that may be different among the sites. Fig. 6 does not show the points for Waterman Road, which had values that were outliers in this figure, with *x*-axis values for PC1 scores of 6–8. However, the regression relationship of the PCs with soil nitrate was not improved by excluding the Waterman Road site, and it is included in the regression analysis (Table 2). The PC scores for *A. californica* and *B. madritensis* FAME profiles were not different except at the Motte Reserve, so they are not shown separately in Fig. 6. This is in contrast to the distinct species separation for fungal colonization variables (Fig. 4b). The FAME profiles and

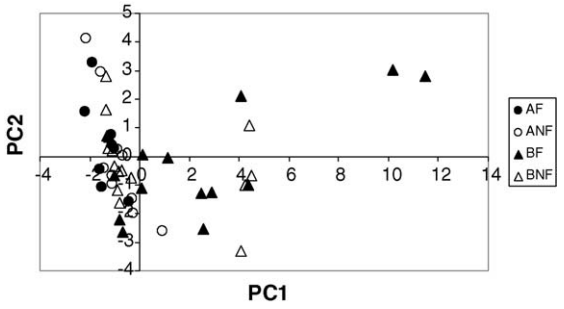

Fig. 7. PCA of soil fatty acids from the N-fertilized plots. The first four PCs were significant according to the broken stick eigenvalue, and all the PCs were significantly related to host plant species but not soil N concentration (Table 2D). A: *A. californica*, B: *B. madritensis*, F: fertilized, NF: nonfertilized.

the PCA of the 1999 samples were similar in trends to the 1998 samples and are not shown here (Sigüenza, 2000).

Twenty-five fatty acids were extracted from soils in the fertilizer experiment at Lake Skinner. Fertilization decreased the abundance of the 16:1ω5c fatty acid in samples from the exotic grasses, but not significantly so for *A. californica* ($p < 0.0001$, Table 3). The other abundant fatty acids, 16:00 and 18:1ω9c were not affected by fertilization.

For the PCA of FAME in the fertilized plots, 53.9% of the variance was explained by the first four PCs (Fig. 7, Table 2). Twenty-four fatty acids were used in the PC analysis. The two-way ANOVA showed a significant effect of host plant on the scores for all four PCs, but no significant effect of N fertilizer on any of the four PCs (Table 2).

4. Discussion

4.1. Root colonization and arbuscular mycorrhizal spores

We detected several patterns of microbial response to invasive grasses and elevated soil N. Decrease of AM colonization with elevated N was previously observed in shrubs along this gradient (Egerton-Warburton and Allen, 2000), and has been observed in grasses and monocots in other studies (Thompson, 1986; Baath and Spokes, 1989). Percent AM colonization is a composite measure of the interaction of two organisms that may respond differently to environmental changes (Allen, 2001). Both *A. californica* and *B. madritensis* may be limited by N in the field, as they responded to N applications by increased growth (Padgett et al., 1999). If the fungus has a lower growth rate than the roots, then percent colonization would decrease. This may explain the response of *A. californica*, but not of *B. madritensis*. This grass was colonized primarily by a fine endophyte that was not reduced by elevated N as was the coarse endophyte. The fine endophyte is most likely *Glomus tenue,* which has been observed primarily in grasses (Rabatin, 1979; McGonigle and Fitter, 1990; Thomson et al., 1992). A more recent study mentions the possibility of more than one species producing fine hyphae (Thippayarugs et al., 1999).

It is not clear whether the fine endophyte is indigenous to California, or originated in the Mediterranean and was introduced with the invasive grasses. In another study on the interactions of an invasive species with mycorrhizal fungi, the fungi were presumed to be indigenous; the exotic *Centauria maculosa* had greater response to mycorrhizal inoculation from a field site in Montana than a native grass (Marler et al., 1999). However, even in that study the origin of the mycorrhizal fungi cannot be certain, until they have been probed with molecular markers. In our study, exotic grass invasion of new habitat may be enhanced by symbiosis with the fine endophyte. Whether native or not, the fine endophyte is known to promote positive growth responses by several grass species (Powell and Daniel, 1978; McGee, 1985).

The responses in the N-fertilized plots were more consistent than in elevated-N sites along the gradient. Although we selected the gradient sites to be as similar as possible, prior analyses showed differences in other soil nutrients, organic matter, and texture (Padgett et al., 1999), but these did not vary in any pattern that could explain increased or decreased colonization at a particular site. Slight changes in site phenology due to differences in elevation, or slight differences in amount of precipitation delivered by any one rain event, could be just as important in controlling % colonization at any site. For instance, fertilization caused a decrease in total AM colonization, vesicles and coils of *A. californica* at Lake Skinner, although these varied along the gradient. In contrast, the fine endophyte of *B. madritensis* did not respond to elevated N. Consequently, the PCA of colonization variables along the gradient had a weak, although significant relationship to soil nitrate. Mycorrhizal responses to fertilization were also dependent on plant species in a study on annual forbs, with some having increased, and others decreased colonization (Rillig et al., 1998).

Patterns of reduced spore density with elevated N were also not clear along the gradient, but the pattern was obvious in the N-fertilization experiment. In a previous study along this gradient, but with multiple observations over the year, spore density was lower in

shrub rhizospheres in the high-N sites (Egerton-Warburton et al., 2000). In the N-fertilization study at Lake Skinner, spore density was higher for *A. californica* in the unfertilized treatment, but the spore density in *B. madritensis* was not affected by N fertilization. This agrees with the colonization observations. An exception to low spore numbers and high soil N was observed at Mockingbird Reservoir. This site had a higher number of small spores (<20 μm) in the grass rhizosphere than other sites. These spores may be *Glomus tenue*, but their identification was not conclusive.

Grasses also had higher colonization by nonmycorrhizal fungi than the shrub. Asymptomatic root pathogens are known to affect plant growth and competition (Newsham et al., 1994). However, it is not clear if the nonmycorrhizal fungi observed were pathogens, as plants exhibited no symptoms such as necrosis or wilt. Using DNA analysis, Vandenkoornhuyse et al. (2003) identified 49 different fungal sequences in roots, only seven of which were AM fungi. These nonmycorrhizal fungi are apparently ubiquitous, but we know little about their functioning. Nonmycorrhizal fungi had increased colonization with Hoagland's nutrient solution and elevated CO_2 (Klironomos et al., 1996), but in our study they were primarily associated with grasses, not with elevated N. The low nonmycorrhizal colonization of grasses at the Lake Skinner site is more difficult to explain, although we did observe higher grass colonization by nonmycorrhizal fungi in a greenhouse bioassay using Lake Skinner soil as inoculum (Sigüenza et al., 2006).

4.2. FAME profiles

The FAME profiles showed another perspective on changes in the soil communities along the gradient and in the N-fertilized plots. The PCA from the N-fertilized plots showed that the exotic grasses had FAME profiles distinct from the native shrub, regardless of N level. Nonnative annual grasses also had distinct microbial fatty acid profiles compared to native species in another study (Steenwerth et al., 2002). Nitrogen fertilization promoted a further separation of the grass FAME profiles in our study, as the most abundant fatty acid declined in the grass although not in the shrub rhizosphere. However, the host plants along the gradient were not differentiated in their FAME profiles, except at one of the intermediate-N sites. This is difficult to explain, unless the timing of application of the N fertilizer at the beginning of the growing season causes a burst in growth of some microorganisms with a distinct FAME profile. In other words, the N-fertilized plots received their N as a pulse rather than daily dry deposition.

Among the most abundant fatty acids we observed, 16:1ω5c is considered a biomarker for arbuscular mycorrhizal fungi, although this fatty acid is also found in bacteria (Olsson, 1999). The biomass of arbuscular mycorrhizal structures may be the largest fraction of the soil microbial biomass (Olsson et al., 1999) and spores may account for 90% of the external fungal biomass (Olsson and Johansen, 2000). So, the results observed in this study are likely more related to the AM fungi than to bacteria, although bacterial fatty acids were also present. However, in our samples none of the fatty acids associated with bacteria were as abundant as those associated with AM fungi. The fatty acids that are known to be bacterial had concentrations one or two orders of magnitude lower than the most abundant ones extracted from these soils.

The concentration of the 16:1ω5c fatty acid was generally higher in the intermediate to low-N sites of the gradient. The decrease of the AMF biomarker 16:1ω5c, due to increased N availability, was more evident in the fertilized plots than along the gradient. Mockingbird Reservoir, a high-N site, also had high levels of 16:1ω5c, coupled with a spore density that was unusually high compared to the other high-N sites. The unique FAME profile of the Waterman Road site may be due to its location at the ecotone between CSS and chaparral and its higher elevation. The most abundant fatty acid at Waterman Road, 19:0 10 methyl, which may be a biomarker for actinomycetes (Brennan, 1988), was not present in the other CSS samples. The other abundant fatty acid in this study, 16:00, was not affected by fertilization. This fatty acid may occur in AMF (Graham et al., 1995; Bentivenga and Morton, 1996; Olsson, 1999), bacteria (Wilkinson, 1988; O'Leary, 1988), actinomycetes (Brennan, 1988), plants and algae (Harwood and Russell, 1984).

4.3. Conclusions

Increased N availability had varied effects on measures of arbuscular mycorrhizal and nonmycorrhizal fungal abundance. Overall, nitrogen fertilization in the experimental plots caused more consistent effects on AM fungi than did N deposition along the gradient. Mycorrhizal colonization of the coarse endophyte of *A. californica* was lower in most higher N deposition sites and in the N-fertilized plots, as has been found in other studies (Bardgett et al., 1999a). The fine endophyte colonized primarily exotic annual grass roots and was

not affected by N. FAME analyses showed that some of the most abundant fatty acids in this study were arbuscular mycorrhizal fungi biomarkers.

If we are to understand the functional implications of these microbial changes, the impacts of changed microbial communities need to be determined by measuring responses of the vegetation to microorganisms (Wardle et al., 1999). In other studies, N-eutrophied mycorrhizae were less mutualistic in promoting growth of native host plants (Johnson, 1993; Corkidi et al., 2002), but response of invasive host plants to N-eutrophied inoculum was not observed in these studies. Still other studies have shown that invasive species harbor microbial communities that promote their growth more than that of the native species (Marler et al., 1999; Zabinski et al., 2002). Functional response studies of the inoculum from these sites showed that *B. madritensis* had a positive growth response to fine endophyte even under elevated soil N, while the coarse endophyte caused growth depressions in *A. californica* in high-N soils (Sigüenza et al., 2006). In addition, *Bromus madritensis* takes up N at a faster rate than *A. californica*, which is meditated by its mycorrhizal fungi (Yoshida and Allen, 2001, 2004). Thus the interactions of inoculum affected both by N and by invasive species may in part explain why *B. madritensis* has become such a widespread invader in southern California.

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