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Reconstruction of the historical changes in mycorrhizal fungal communities under anthropogenic nitrogen deposition

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Archived soil samples (1937–1999) and historic air quality data from the Los Angeles Basin were used for reconstructing the record of change between atmospheric NO_x loads, soil $\delta^{15}N$ values and the diversity of arbuscular mycorrhizae (AM), which are ubiquitous plant–fungus mutualists that control plant community productivity. A tripling of atmospheric NO_x loads between 1937 and the 1970s was paralleled by soil nitrogen enrichment ($\delta^{15}N = 3.18$). From 1975 onwards, atmospheric NO_x declined, but soils became nitrogen saturated ($\delta^{15}N = -4$ and NO_3-nitrogen $= 171\ mg\ kg^{-1}$). The shifts in the AM community followed 28 years of atmospheric nitrogen enrichment and coincided with the onset of soil nitrogen saturation. Such changes were manifest in the loss of AM productivity, species richness (one species per year), three genera (*Acaulospora*, *Scutellospora* and *Gigaspora*) in the spore community and *Gigaspora* within the roots. Nitrogen enrichment also enhanced the proliferation of potentially less mutualistic species of *Glomus*. Autoregressive models implied that such patterns will persist and be driven by soil nitrogen cycling patterns. Chronic nitrogen enrichment from air pollution thus alters the diversity and mutualistic functioning of AM communities, which, in turn, may influence the plant community.

Keywords: arbuscular mycorrhizae; diversity; nitrogen saturation; community variation

1. INTRODUCTION

Seventy per cent of all global NO_x emissions are the result of human activities (Fowler *et al*. 1998). One of the consequences is an enhanced nitrogen input into terrestrial ecosystems (Vitousek *et al*. 1997; Fenn *et al*. 1998). Nitrogen enrichment causes a decrease in plant species' diversity, shifts in community composition and species' dominance and the invasion of non-native species (Berendse 1990; Bobbink 1991; Wedin & Tilman 1996). Furthermore, a direct correlation exists between nitrogen enrichment and a decline in the diversity of mycorrhizae, which are plant–fungus mutualists (Johnson *et al*. 1992; Egerton-Warburton & Allen 2000). Alterations in mycorrhizal dynamics have the capacity for modifying plant community dynamics based on the causal relationship between mycorrhizal fungal diversity and plant species' diversity (Van der Heijden *et al*. 1998). The actual sequence may thus be that nitrogen enrichment induces shifts in the mycorrhizal fungal community, which, in turn, initiate changes in the plant community. However, the foundation for interpretation and prediction of this chain of events is largely built on observations of contemporary patterns or empirical data from fertilization experiments (Heijne *et al*. 1992; Johnson 1993; Egerton-Warburton & Allen 2000).

Here, we present the alternative to a historical perspective and reconstruct the record of change in a shrub community in the Los Angeles Basin (1937 to 1999) between atmospheric NO_x loads, soil nitrogen ($\delta^{15}N$) and the diversity of arbuscular mycorrhizae (AM), which are ubiquitous mutualists that control plant community diversity and productivity (Van der Heijden *et al*. 1998). The Los Angeles Basin has had a history of trapping air pollutants since the 1940s. Burgeoning urban expansion in combination with increased combustion of fossil fuels (Alexis *et al*. 1999) has contributed to increasing atmospheric NO_x loads (figure 1*a*) and some of the most extreme air pollution in the contiguous USA (Bytnerowicz & Fenn 1996). Elevated atmospheric NO_x loads have resulted in a soil nitrogen enrichment that corresponds to an annual input of 33–38 kg nitrogen ha^{-1} (or 10–13% emissions) deposited primarily as nitrates (Bytnerowicz & Fenn 1996; Fenn *et al*. 1998). In comparison, nitrogen enrichment in Europe is primarily associated with ammonium deposition from agricultural sources (Heil *et al*. 1988). In addition, ozone (O_3) co-occurs with NO_x in southern California (Miller *et al*. 1998) and may confound the effects of nitrogen (Grulke *et al*. 1998). However, the phenology of shrubs and their mycorrhizae is offset from the timing of peak O_3 levels (Westman 1981; Miller *et al*. 1998) and, thus, the effects of O_3 are considered to be relatively small (Allen *et al*. 2000).

We evaluated the long-term correlates of anthropogenic nitrogen pollution by examining soil $\delta^{15}N$ values and the diversity of AM fungal spores in preserved soil samples (1937–1999) and extant mycorrhizae on root samples (1999) from the San Dimas Experimental Forest, one of the most heavily polluted sites within the Los Angeles Basin. Such circumstances are analogous to over-fertilization of agricultural lands (Tilman *et al*. 2001) and are potentially predictors of the time-sequence and levels at which nitrogen enrichment may become environmentally and ecologically significant.

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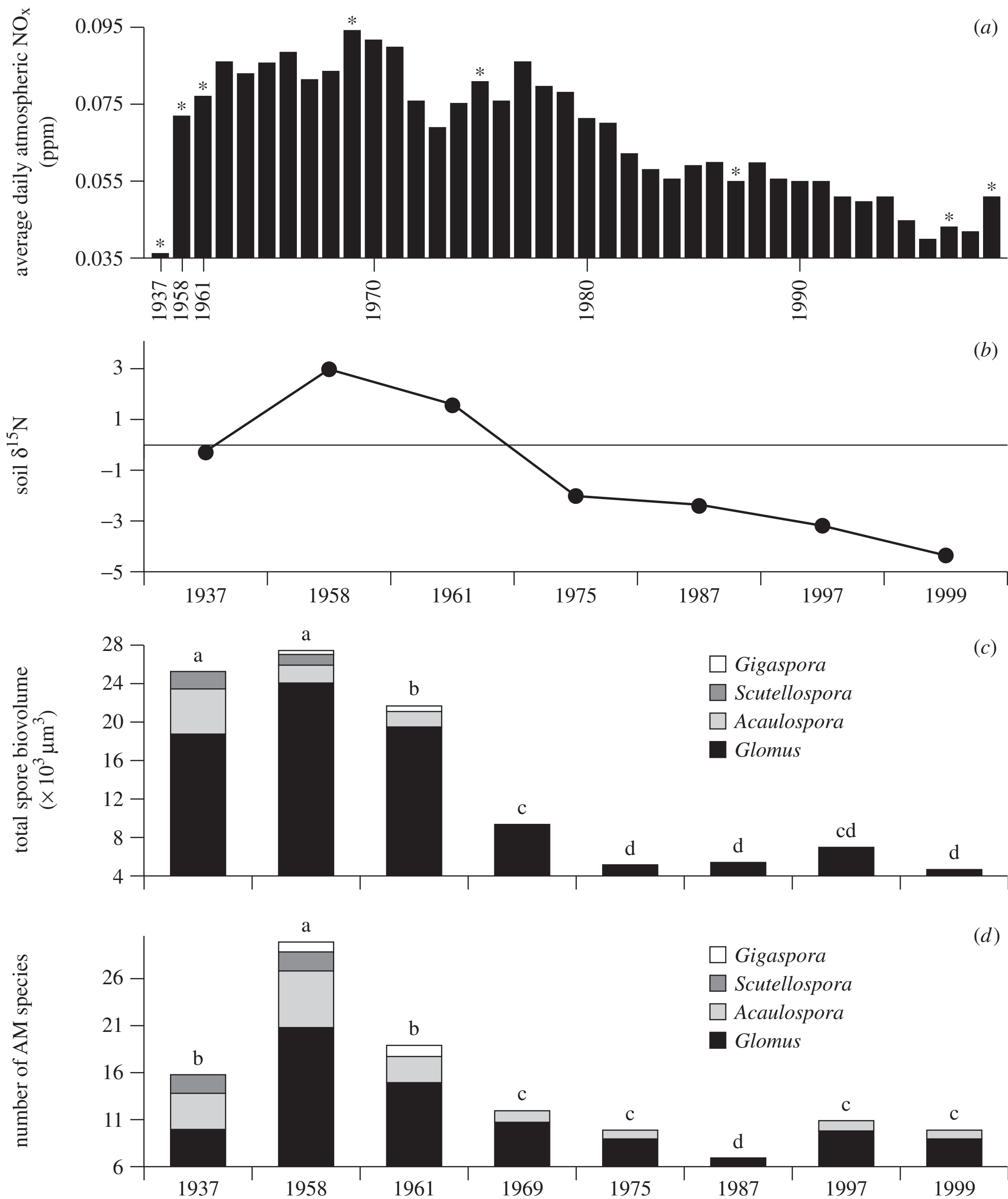

Figure 1. (*a*) Atmospheric NO_x loads in the Los Angeles Basin from 1937 to 1999. An asterisk over a column indicates the years in which soil samples were analysed for $\delta^{15}N$ and mycorrhizae. The data are presented as annual mean concentrations of NO_x due to the difficulty in determining the precise rate of atmospheric nitrogen deposition in forests (Fenn & Bytnerowicz 1993). Temporal changes in (*b*) soil $\delta^{15}N$ values, (*c*) spore biovolume and (*d*) arbuscular mycorrhizal species richness in archived soil samples from 1937 to 1999. Mean values with the same letter do not differ significantly at $p < 0.05$.

2. MATERIAL AND METHODS

(a) *Site description and sampling procedures*

Preserved soil samples were analysed from collections undertaken at unconfined lysimeters in the San Dimas Experimental Forest (34°06′ N, 117°48′ W and elevation 830 m), which is a United States Department of Agriculture Pacific Southwest Forest Services' research facility that is situated within the Los Angeles Basin. The region is typified by a warm Mediterranean-type climate (mean annual temperature 14.4 °C) and, on average receives 678 mm precipitation per annum (Dunn *et al.* 1988). The soils within the basin are composed of sandy loams or sandy clay loams that are derived from a weathered granite or granodiorite (United States Department of Agriculture 1971), tend to be slightly acidic (pH 5.8–6.0) and contain significant reserves of phosphorous (mean 30 $\mu g\, g^{-1}$, HCO_3-extractable) and potassium (200 $\mu g\, g^{-1}$). The topographical and meteorological conditions at San Dimas are conducive to the trapping of pollutants and promote both dry (summer) and wet nitrogen deposition (spring fog) (Bytnerowicz & Fenn 1996).

The lysimeters were constructed in 1937, overfilled with homogenized fine sandy loam (collected to a depth of 2.1 m) and allowed to settle until 1940. The lysimeters were seeded with *Bromus mollis* L. from 1940 until 1945 in order to develop the soil

profile, after which monocultures of *Eriogonum fasciculatum* var. *foliolosum* Nutt., *Adenostoma fasciculatum* Hook. & Arn., *Ceanothus crassifolius* Torrey, *Quercus dumosa* Nutt. and *Pinus coulteri* D. Don were established using seedlings of each species (Patric 1961). By the mid-1950s, the lysimeters supported vigorous and virtually pure stands of shrubs that were similar in size, density and reproductive status to those in natural stands (Patric 1961). For the most part, the stands have persisted as such to the present day. These conditions promoted near constancy of the soil parent material, topography and above-ground biota that eliminated potentially confounding covariates in the analysis of mycorrhizae, but still promoted active paedogenesis (e.g. Ulery *et al.* 1995).

Formal soil sampling commenced in all lysimeters in 1958. Soils from the lysimeters planted with *E. fasciculatum* var. *foliolosum* and *A. fasciculatum* were assayed for the purposes of this study, as these plant species generally support diverse AM communities in natural shrub lands. The soils retained from the homogenized fill were representative of 1937. The remaining soil samples were collected by coring to a depth of 10–15 cm at the drip line (1958–1975 and 1999) or just under the canopy of shrubs (1987–1997), air dried and stored in sealed containers in the laboratory at ambient temperature (16 °C) under dark conditions. Such processing and storage conditions effectively preserved the mycorrhizal spores as more spores were recovered from archived 1958 soils than those collected in 1999 (221 versus 42 spores per gram of soil, respectively). If spore senescence had occurred with storage, the densities would be higher in 1999 than in previous years. A subsample from the 1999 collection was retained for colorimetric nitrogen analysis and examination of the roots.

(b) *Soil nitrogen and pH status*

The nitrogen status of the soils from 1937 to 1999 was evaluated by the soils' natural abundances of nitrogen isotopes ($\delta^{15}N$) as the $\delta^{15}N$ of soils can be differentially influenced by nitrogen components from atmospheric deposition, fertilization or precipitation (Bauer *et al.* 2000). Soil ^{15}N:^{14}N ratios were acquired using a Europa Scientific Hydra 20/20 continuous flow isotope ratio mass spectrometer (± 0.3 reproducible accuracy; University of California, Davis). The isotopic composition was calculated as the relative difference in the ^{15}N:^{14}N ratio between a sample (R_{sample}) and atmospheric nitrogen standard (0.0036765) as follows:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000‰. \quad (2.1)$$

Soils collected in 1999 were extracted in 2 M KCl and analysed using a continuous flow analyser for NO_3 (cadmium reduction method) while the pH of each sample was determined in a paste of soil in 0.01 M $CaCl_2$.

(c) *Assaying the arbuscular mycorrhizal community*

Arbuscular mycorrhizal spores were extracted and enumerated from each of three replicate soil samples per year and plant species using the methods detailed in Egerton-Warburton & Allen (2000). Spores were mounted in 1:1 polyvinyl alcohol:Melzer's reagent, examined under a light microscope ($\times 400$–1000) equipped with differential interference contrast optics and identified to species level on the basis of wall morphology in comparison with authenticated type samples held in a reference collection or published descriptions. Spores not identified to species level were allocated an identifier by genus and number. Representative slides are held in permanent storage at The University of California, Riverside.

Extant mycorrhizae were assayed by direct immunofluorescence of root samples collected in 1999. Fine roots were washed from soil cores and incubated in fluoroscein-conjugated antisera that were raised against the spores of each of the four major AM genera (*Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora*) (detailed in Egerton-Warburton & Allen 2000). The samples were assessed for the percentage of their root length that was colonized by extra-radical (vegetative) hyphae of each AM genus.

(d) *Statistical analyses*

All data and analyses represent the pooled data of *E. fasciculatum* and *A. fasciculatum* due to the limited replication at each sampling date. Species richness was expressed as the number of AM species recovered per year (S), and differences between sampling times were analysed by χ^2-statistic analysis (Zar 1988). Species turnover (I_y) was calculated using the regression of $\ln(I)$ against $\ln(\text{time})$ as follows:

$$I = 100 \times (E + H)/(C + D), \quad (2.2)$$

where E is the number of AM species gained, H is the number of species lost, C is the number of species present in the first survey and D is the number present in the second or subsequent surveys (Lynch & Johnson 1974).

Changes in the productivity and functional importance of the species and genera within the community were represented by the spore biovolume (V_b), that is a composite measure of spore abundance and species identity. Spore volumes were calculated for each species using the equation for a prolate spheroid. The total spore biovolume per species was calculated by multiplying the spore volume by its relative abundance (RA) in the community. Relative abundance was calculated as

$$\text{RA} = (n_i/n_t) \times 100, \quad (2.3)$$

where n_i is the number of spores of an individual AM taxon and n_t is the total number of AM spores examined in the sample. In addition, spore dilution effects were taken into account when calculating the value of V_b for 1937 because the sampling procedures adopted in 1937 (collections to 2.1 m depth) differed from those in other years (collections to 15 cm depth) and most AM spores are concentrated in the top 10–15 cm of the soil profile. Differences in the relative abundance and spore biovolume were analysed using one-way ANOVA and further analysed by Fisher's least-significance difference tests (Zar 1988).

Time-series analysis was used to establish the general model of species' abundance over time and detecting AM taxa that deviated from the overall model. Log_e-transformed sample dates and the relative abundance data of each AM species and sample time were analysed by the Blackman–Tukey method (95% confidence interval) in the AnalySeries package (www.ngdc.noaa.gov/paleo/softlib.html). The datasets were analysed further using an autoregressive model of mycorrhizal species richness in order to draw inferences about the relative contribution of air pollution and soil nitrogen enrichment on mycorrhizal dynamics over time. Forward, linear, stepwise regression was used for modelling coefficients as a function of atmospheric NO_x, soil nitrogen status and mycorrhizal species richness. Intercepts with significant contributions to the model were retained. Unless otherwise noted, all statistical analyses were undertaken on SAS v6.01 (SAS Institute, Cary, NC, USA).

3. RESULTS AND DISCUSSION

The sequence of change in air and soil nitrogen from the 1930s to the turn of the century is recorded in atmospheric NO_x loads and soil $\delta^{15}N$ values (figure 1*a*,*b*). A tripling of

atmospheric NO_x loads between 1937 and 1970 was paralleled by soil nitrogen enrichment (figure 1*b*) that, based on $\delta^{15}N$ values, was probably derived from industry, cars and the diesel buses used for replacing mass electric transit in the 1960s (Bauer *et al*. 2000; www.arb.ca.gov).

The 1970s onwards were marked by a decline in atmospheric NO_x due to the closure of heavy industry (e.g. steel processing) in Los Angeles, new emission controls for cars and a legislative framework that mandated deadlines for meeting clean air standards (Federal Clean Air Act 1970 *et sequens*; www.epa.gov). However, during this period soils demonstrated a shift to depleted (negative) $\delta^{15}N$ values and were nitrogen enriched (NO_3-nitrogen $= 171\ mg\ kg^{-1}$). Taken together, these data denote that nitrogen availability had increased beyond the capacity of the system for storing or cycling nitrogen internally and, in turn, the onset of soil NO_3- nitrogen saturation within the lysimeters (Aber *et al*. 1989; Högberg 1997). Nevertheless, anthropogenic acidification was not associated with nitrogen enrichment at San Dimas since the soil pH increased from 5.50 to 7.10 between 1937 and 1999. In addition, atmospheric sulphur deposition within the region was sufficiently low (one-tenth the input of nitrogen) as to not cause acidification events (Fenn & Bytnerowicz 1993). Thus, any observed shifts in mycorrhizal communities were probably attributable to soil nitrogen enrichment alone and such effects may persist even when the air quality has seemingly improved.

Dynamic changes in the AM community accompanied the temporal sequence of nitrogen enrichment (figure 1*c*,*d*). The AM community was both productive and diverse from the inception of the lysimeters until 1958 (figure 1*c*,*d*), even with constant nitrogen enrichment (figure 1*b*). During this time, nitrogen was most probably cycled in a closed system: nitrogen taken up by plants was allocated to tissues, lost as litter, mineralized and used by plants, leaving the enriched fraction in the soil (Högberg 1997). However, the era between 1958 and 1997 was characterized by a progressive decline in the mycorrhizal community (figure 1*c*,*d*). The significant loss of taxa commenced around 1969 and the threshold for change was allied with the onset of soil nitrogen saturation (figure 1*b*).

Nitrogen enrichment was associated with the decreased productivity (figure 1*c*) ($F_{7,20} = 9.31$ and $p < 0.0001$) and replacement of a formerly diverse AM community ($n = 29$ species) with one composed of only seven taxa; such changes equated to the loss of one species per year ($I_y = -1.13$) (figure 1*d*). Notably, species of the larger-spored genera *Acaulospora*, *Scutellospora* and *Gigaspora* and many *Glomus* species disappeared completely from the community (figure 1*d* and table 1) (*Scutellospora*, *Acaulospora* and *Gigaspora* combined χ^2_8, $p = 0.0001$ and *Glomus* χ^2_8, $p = 0.0001$). Experimental nitrogen fertilization also produces the same effects on the AM community in native shrub lands (Egerton-Warburton & Allen 2000) and, thus, nitrogen input rather than other environmental factors within the lysimeters *per se* may explain the causal relationship between nitrogen enrichment and shifts in the AM community.

While the overall pattern was one of declining abundance for most AM species, time-series analysis indicated two notable exceptions to this trend: *Glomus aggregatum* and *Glomus leptotichum* increased significantly in abundance over the temporal sequence (table 1). These species may be indicative of enrichment following nitrogen fertilization (Johnson 1993) and anthropogenic nitrogen deposition (Egerton-Warburton & Allen 2000) and may be affiliated with colonization by invasive grasses in southern California (Nelson & Allen 1993). In symbiosis they sometimes exert a net negative carbon balance on the host that is analogous to a parasitic rather than mutualistic association (Johnson 1993). Correspondingly, the loss of diversity and selection of functionally distinct taxa may impact on obligate mycorrhizal species, rhizosphere flora and below-ground food webs and, in turn, ecosystem stability and functioning as a whole.

An absence of spores does not necessarily mean that a genus has disappeared from the AM community (Egerton-Warburton & Allen 2000). Immunofluorescence of root samples in 1999 revealed that *Glomus* (31% root length infected), *Acaulospora* (25% root length infected) and *Scutellospora* (1.2% root length infected) but not *Gigaspora* persisted in a vegetative (hyphal) form in 1999 even though spores of *Scutellospora* were not present in the soil samples (table 1). The absence of *Gigaspora* within the spore community and in vegetative form suggests the loss of the genus from the AM community around 1969 (figure 1*c*,*d*) and that *Gigaspora* was more sensitive to nutrient-enriched conditions than the other AM genera (Douds & Schenck 1990; Johnson 1993).

The conclusion of the temporal sequence (1997–1999) was notable for a small but significant increase in species richness and the renaissance of certain species (e.g. *Acaulospora mellea*, *Glomus mosseae* and *Glomus* aff. *scintillans*), but no substantial increase in recruitment (figure 1*c*,*d* and table 1). Such increases coincide with increasingly stringent vehicle emission standards in California, a concomitant reduction in atmospheric NO_x and the El Niño weather pattern, which produces atmospheric conditions that inhibit smog formation (figure 1*a*) (Alexis *et al*. 1999). More interestingly, these data indicate a degree of resilience in the AM community and the capacity to recover from nitrogen enrichment. Empirical and descriptive studies indicate that recovery is typically protracted and that only small guilds of fungi can be expected to return, particularly in formerly diverse AM communities (Allen *et al*. 1993). Nevertheless, we hypothesize that this renaissance may be short-lived since soil nitrogen cycling ($r = 0.60$ and $p = 0.032$) will continue to influence AM species richness (overall model $R^2 = 0.648$).

Accordingly, we propose that the cumulative effects of anthropogenic nitrogen enrichment may profoundly influence the AM community and that these changes may determine ecosystem responses to elevated nitrogen deposition through a series of negative feedback loops. Contemporary studies demonstrate the causal nature of this relationship. Nitrogen fertilization selects for less effective mutualists (Johnson 1993). Nutrient enrichment also promotes shifts in the quality and quantity of plant carbon allocated to the mycobiont (Douds & Schenck 1990), the biomass of fine roots available for colonization (Grulke *et al*. 1998) and the abundance of intra- and extra-radical mycorrhizal structures that supply water and nutrients to the host plant (Egerton-Warburton & Allen 2000). In turn, such changes may impact negatively on host plant productivity, nutrient capture and competitive ability

Table 1. Relative abundances of arbuscular mycorrhizal taxa from 1937–1999 at the San Dimas Experimental Forest.

(The data represent pooled analyses of *E. fasciculatum* var. *foliolosum* and *A. fasciculatum*. The relative abundances in the significance column differ at $^{*}p < 0.05$, $^{**}p < 0.01$ or $^{***}p < 0.001$ or were not significantly different (n.s.) ($p > 0.05$); means within rows with the same letter do not differ at $p < 0.05$. n.a., analyses not applicable (less than three sample points in the temporal sequence).)

	year								
AM taxa	1937	1958	1961	1969	1975	1987	1997	1999	significance
Acaulospora mellea	10.26^{a}	2.67^{b}	3.70^{b}	1.98^{c}	0.85^{c}	—	0.85^{c}	1.29^{c}	***
Acaulospora laevis	—	0.53	—	—	—	—	—	—	n.a.
Acaulospora tuberculata	1.28	0.26	—	—	—	—	—	—	n.a.
Acaulospora elegans	3.70	—	—	—	—	—	—	—	n.a.
Acaulospora scrobiculata	1.28	0.24	—	—	—	—	—	—	n.a.
Acaulospora spinosa	—	0.24	0.53	—	—	—	—	—	n.a.
Gigaspora margarita	—	0.29	0.53	—	—	—	—	—	n.a.
Scutellospora calospora	1.28	0.89	—	—	—	—	—	—	n.a.
Scutellospora fulgida	1.28	0.23	—	—	—	—	—	—	n.a.
Glomus aggregatum	44.87^{c}	54.60^{c}	67.20^{b}	59.41^{b}	74.53^{a}	72.73^{a}	58.47^{b}	69.74^{ab}	***
Glomus clarum	1.28^{b}	2.67^{b}	3.70^{a}	2.97^{b}	—	—	0.43^{b}	—	*
Glomus claroideum	—	1.48	1.06	0.99	—	—	—	—	n.s.
Glomus deserticola	—	5.64^{a}	—	1.41^{b}	—	1.30^{b}	—	2.58^{b}	*
Glomus etunicatum	—	1.19^{c}	2.12^{c}	8.64^{a}	0.85^{c}	8.15^{a}	6.78^{ab}	7.30^{a}	***
Glomus fasciculatum	—	0.30^{c}	1.06^{b}	2.97^{a}	—	1.30^{b}	—	1.93^{b}	n.s.
Glomus geosporum	3.85^{a}	4.15^{a}	1.59^{b}	1.98^{b}	0.62^{c}	0.92^{c}	0.85^{c}	1.50^{b}	**
Glomus leptotichum	—	0.29^{c}	2.65^{c}	1.41^{c}	1.86^{c}	6.50^{a}	9.32^{a}	3.43^{b}	***
Glomus macrocarpum	—	2.12	—	—	—	—	0.85	—	n.s.
Glomus microaggregatum	—	3.56	—	—	—	—	—	—	n.a.
Glomus mosseae	1.28	0.89	—	—	—	—	—	3.22	n.s.
Glomus occultum	1.28^{d}	2.37^{c}	4.76^{b}	4.95^{b}	15.53^{a}	9.10^{a}	15.25^{a}	3.00^{bc}	***
Glomus spurcum	12.82^{a}	5.04^{b}	1.06^{c}	3.96^{c}	1.86^{c}	—	0.21^{d}	—	**
Glomus tenue	1.28^{c}	6.53^{a}	2.65^{b}	3.96^{b}	3.26^{b}	—	5.93^{a}	4.94^{a}	***
G. aff. *scintillans*	—	1.06^{b}	3.96^{a}	—	—	—	—	0.43^{b}	*
Glomus sp. 1	12.97^{a}	3.86^{b}	0.53^{c}	—	0.62^{c}	—	1.69^{c}	—	**
Glomus sp. 2	1.28	1.19	—	—	—	—	—	—	n.a.
Glomus sp. 3	—	0.30	0.53	—	—	—	—	—	n.a.
Glomus sp. 4	—	1.06	1.41	—	—	—	—	—	n.a.
Glomus sp. 5	—	0.59	1.06	—	—	—	—	—	n.a.
Glomus sp. 6	—	0.30	—	—	—	—	—	—	n.a.

(Sanders & Fitter 1992; Van der Heijden *et al.* 1998) and potentially alter phosphorus cycling and carbon allocation at the ecosystem level (Zak *et al.* 1993). By the same token, changes in carbon allocation may modify the capacity of terrestrial ecosystems for absorbing and sequestering carbon dioxide-derived carbon, particularly under the projected scenarios of global change (Schindler & Bayley 1993; Schimel 1995; Townsend *et al.* 1996).

Variations in mycorrhizal community diversity may also impact directly on plant community structure and diversity (Van der Heijden *et al.* 1998). These consequences may have already occurred within the Los Angeles Basin, but no historic record exists to enable us to evaluate the mechanisms of plant community change with nitrogen enrichment at San Dimas. Nevertheless, contemporary data suggest that such changes may have already occurred in southern California (Westman 1981; Minnich & Dezzani 1998) and elsewhere (Aber *et al.* 1989; Schulze 1989; Fenn *et al.* 1998) on a landscape scale and, hence, may foreshadow continuing changes in the industrialized world. Air pollution in Phoenix, Houston and Tucson may soon match the magnitude recorded within the Los Angeles Basin. Similarly, the dramatic increase in the combustion of fossil fuel in economically developing countries may contribute to the globalization of nitrogen deposition and eutrophication (Matson *et al.* 1999). We anticipate that there are similar changes occurring within these mycorrhizal communities and that they will ramify through the ecosystem.

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