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Changes in soil microbial and nematode communities during ecosystem decline across a long-term chronosequence

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Abstract

Following the creation of new land surfaces, there is an initial build-up phase, but in the prolonged absence of catastrophic disturbance an ecosystem decline phase has often been observed. While a number of studies have investigated the changes in soil biota that occur during the build-up phase, few studies have investigated how the soil food web changes during the ecosystem decline phase, even though such studies may assist our understanding of biotic factors that contribute to long-term ecosystem changes. We investigated the response of soil microbial and nematode communities to ecosystem decline by studying each of four stages of a long-term (280,000 year) forested chronosequence caused by uplift of marine terraces in the Waitutu region of Fiordland National Park, New Zealand. With increasing chronosequence age there were large increases in ratios of C to N, C to P, and N to P in both the organic layer and mineral soil layer, indicative of greater nutrient (notably P) limitation over time. Variables related to soil microbial biomass and activity were lower on the older terraces when expressed on a per unit soil C basis, reflecting that the quality of the soil organic matter, which is the resource that supports microbial metabolism, declined over time. This in turn had important consequences for population densities of soil nematodes and enchytraeids, including both microbe-feeding and predatory groups. There were significant increases in the fungal:bacterial biomass ratio and in the fungal-feeding:bacterial-feeding nematode densities. Taken collectively, our results suggest a decline in soil microbial activity and soil fauna, and an increase in the relative importance of the fungal-based (vs. bacterial-based) energy channel during long-term ecosystem development on terraces of marine origin. This corroborates the hypothesis that the studied sites represent a retrogressive shift in soil organic matter quality over a long-term chronosequence.
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1. Introduction

Following the creation of new land surfaces, there is an initial phase of community and ecosystem development during which plant biomass builds up to a maximal biomass phase. This build-up phase has been extensively studied, and involves broadly predictable changes in nutrient accumulation and availability, and above- and belowground ecosystem processes (Clements, 1928; Odum, 1969; Walker and del Moral, 2003). However, in the long-term absence of catastrophic disturbance, an ecosystem decline phase has often been observed, during which there is a long-term reduction in ecosystem productivity and plant biomass (Walker et al., 1983; Crews et al., 1995; Wardle et al., 1997, 2003a). Unlike the build-up phase, the mechanisms driving this ecosystem decline phase are poorly understood and appear to vary from ecosystem to ecosystem, although the decline is often associated with the long-term lack of availability of soil nutrients, notably phosphorous (Walker and Syers, 1976; Vitousek and Farrington, 1997; Hedin et al., 2003).

The activity of the soil biota is critical for regulating the supply of plant-available nutrients from the soil and hence ecosystem productivity. A growing number of studies have

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investigated how densities, biomasses and communities of the soil biota develop during the ecosystem build-up phase across chronosequences. These include studies on the soil microbial biomass (Insam and Haselwandter, 1989), and the community structure of microbes (Ohtonen et al., 1999), saprophytic fungi (Frankland, 1998) and mycorrhizal fungi (Dighton and Mason, 1985). Other studies have focused on groups of soil fauna, such as nematodes (Wasilewska, 1994), microarthropods (Setälä and Marshall, 1994; Chauvat et al., 2003) and earthworms (Scheu, 1992). Conversely, whether groups of soil organisms decline in tandem with the vegetation decline observed during ecosystem decline remains largely unexplored. There is, however, some evidence that during a long-term decline phase there is a concomitant reduction in the soil microbial biomass (Wardle and Ghani, 1995), as well as in the rates of key processes driven by the soil biota such as plant litter decomposition (Crews et al., 1995).

In this study, we sought to assess the changes in the community composition of the soil biota that occur during ecosystem decline by using a long-term established chronosequence. Specifically, we assessed whether changes in soil resource quality showed changes in tandem with general changes vegetation across the chronosequence, and whether these changes were manifested in each of three trophic levels of a soil food web, i.e. soil microbes and herbivorous nematodes (primary consumers), microbe-feeding nematodes (secondary consumers) and top predators (tertiary consumers). To achieve this, we assessed the effects of chronosequence stage on the biomass or total abundance, community composition, and community diversity, of both the soil microbial and soil nematode biota.

2. Methods

2.1. Study site and sampling

The chronosequence that we investigated is located in the Waitutu forest of Fiordland National Park on the southern coast of the South Island, New Zealand (46°06′S, 167°30′E). The rainfall ranges from 1600 to 2400 mm/yr, and the mean January and July temperatures are 12 and 5 °C, respectively. The Waitutu sequence consists of a series of terraces caused by the periodic uplift of marine sediments from the sea floor; these terraces vary in age from 3000 to 600,000 years (Ward, 1988). During the later stages of this chronosequence there is a distinct ecosystem decline with a substantial reduction in vegetation stature on the older terraces; canopy height ranged from 25 to 28 m and total tree basal area of 92 $m^2\,ha^{-1}$ on the recent alluvial terraces to a canopy height of 0–3 m and total basal area of 9 $m^2\,ha^{-1}$ on the oldest stage (Mark et al., 1988; Ward, 1988). The latter stages of the chronosequence represent a typical ecosystem 'regression' (sensu Walker et al., 2001), most likely caused by declining availability of nutrients over time.

For our study, we focused on four stages of the chronosequence, which represented a gradient of increasing age and declining fertility. These are characterised as:

Stage 1: Recent alluvial terraces, formed less than 10,000 years ago. As these terraces are flooded most years, there is still frequent silt deposition. Forested, with dominant canopy species consisting of *Nothofagus menziesii* and *Weinmannia racemosa*, and scattered emergents of *Dacrydium cupressinum* and *Dacrycarpus dacrydioides*. Mean canopy height 25–28 m and total tree basal area 92 $m^2\,ha^{-1}$ (Mark et al., 1988).

Stage 2: Terraces of 80,000 years age. Forest, dominated by *N. menziesii*, *Nothofagus solandri*, *W. racemosa*, *Metrosideros umbellatum*, *Prumnopitys ferruginea* and *D. cupressinum*. Mean canopy height 14–20 m and total tree basal area 82 $m^2\,ha^{-1}$ (Mark et al., 1988).

Stage 3: Terraces of 210,000 years age. Short forest, dominated by *D. cupressinum*, *Podocarpus totara*, *W. racemosa* and *Halocarpus biforme*. Mean canopy height 14–16 m and total tree basal area 75 $m^2\,ha^{-1}$ (Mark et al., 1988).

Stage 4: Terraces of 290,000 years age. Shrubland/short forest, dominated by *H. biformie*, *Halocarpus bidwillii*, *N. solandri* and *Leptospermum scoparium*. Mean canopy height 1–5 m and total tree basal area 23 $m^2\,ha^{-1}$ (Mark et al., 1988).

Within our study area of approximately 40 km^2, we established twenty 20 m × 20 m plots, i.e. five plots within each of the four chronological stages. The five plots at each stage were at least 300 m (and up to 3 km) apart from each other, and they could therefore be considered as true independent replicates. Over December 1–10 2001, soil sampling was performed for all 20 plots. Within each plot, at least 50 random soil cores were collected with a 25 mm diameter soil corer. Plant litter was removed from the soil surface before each core was taken. The cores were then separated into each of two depth layers, i.e. the full depth of the organic layer (termed 'organic layer'), and the top 5 cm of the next soil layer (termed 'mineral soil', since in all but the oldest chronosequence soils contained $<20\%$ organic C). For each plot, the organic layer within each plot was then bulked into a single sample, as was the mineral soil. Each bulked sample was then subsampled as required for individual analytical procedures.

2.2. Soil analyses

A subsample of each soil sample of approximately 100 g (wet wt) was removed for nematode analyses and the remainder of the sample was sieved to <4 mm for soil

chemical and microbial analyses. The following soil chemical analyses were performed: soil pH; total C through the Leco furnace method and infrared detection; total N through Kjeldahl analysis; total P as described by Jackson (1958); bicarbonate-extractable P by the Olsen P method; and ammonium and nitrate through Technicon auto-analysis following extraction with 2 M KCl (Technicon Instruments, 1977).

For each sieved sample, microbial basal respiration (BR) and substrate-induced respiration (SIR), a relative measure of active microbial biomass, were determined as described by Wardle (1993). Briefly, a 10 g (dry weight equivalent) subsample of soil was amended to 90% sieved water holding capacity either by gradual drying or rewetting with a fine mist, placed in a 130-mL airtight vessel, and incubated at 22 °C. Moisture holding capacity was determined as described by Orwin and Wardle (2004); briefly this involved saturating 150 g soil with distilled water and placing it in a covered cylinder that allowed drainage, the moisture content of that soil after overnight drainage was considered to be the sieved water holding capacity (SWHC) of that soil. Evolution of CO_2 between 1 and 4 h were determined in the airtight vessel by injecting 1 mL subsamples of headspace gas into an infrared gas analyser and used as a measure of BR (μg CO_2-C g^{-1} soil h^{-1}). Measurement of SIR (Anderson and Domsch, 1978) was performed in the same way, but with amendment of the subsample with 150 mg glucose powder at the start of the incubation (Wardle, 1993).

Two measures of microbial community composition were made from a sieved subsample of each sample. The first of these involved assessing composition of microbial phospholipid fatty acids (PLFAs) using the method of Bligh and Dyer (1959), as modified by White et al. (1979) and Bardgett et al. (1996); different PLFAs are derived from different subsets of the soil microflora. Briefly, 1.5 g (fresh soil) was extracted using chloroform, methanol and citrate buffer (1:2:0.8 by volume). Extracts were split into two phases using chloroform and citrate buffer, with the chloroform phase containing the lipid fraction recovered. Lipids were fractionated on silicic acid columns (Isolute; 500 mg silicic acid in 6-mL reservoirs), with the phospholipids recovered in methanol, from which fatty acid methyl esters were prepared by mild alkaline methanolysis. The methyl esters were analysed using gas chromatography (GC) on a SGE 25QC3 BP-5 25 m × 0.32 μm film thickness with flame ionisation detector (at 150 °C, 4–250 °C, with helium as a carrier gas at 2.7 mL min^{-1} and auxiliary gases nitrogen at 30 mL min^{-1}, hydrogen at 30 mL min^{-1}, and air at 400 mL min^{-1} for 25 min). The separated methylated fatty acids were identified by chromographic retention time in comparison to bacterial methyl esters standards (Supeloc Bacterial Acid Methyl Esters CP Mix 47080-U) and reference GCMS of fungal isolates to determine the relative retention time of the fungal PLFA. For each soil, the abundance of each of the individual fatty acids extracted was expressed as relative nmoles per gram of dry soil and using standard nomenclature (Tunlid et al., 1989). Representative bacterial PLFAs were i-15:0, a-15:0, 15:0, i-16.0, i-17:0, cy-17:0, 17:0, and cy-19:0, and the PLFA used to represent fungi was 18:2ω6 (Parekh and Bardgett, 2002). In addition to the above PLFAs, 11:0, 2-OH 10:0, 12:0, 13:0, 2-OH 12:0, 3-OH 12:0, 14:0, 2-OH 14:0, 3-OH 14:0, 16:1ω9c, 16:0, 17:0, 2-OH 16:0, 18:1ω9c, 18:1ω9t, 18:0, 19:0 and 20:0 were also used for the calculation of total PLFAs and ordination analyses. The ratio of fungal PLFA to bacterial PLFAs (as specified above) was used as an estimate of the relative importance of the bacterial and fungal energy channels (Bardgett et al., 1996; Parekh and Bardgett, 2002).

The second approach involved characterising microbial communities based on their Substrate Utilisation Profiles (SUPs) (Degens, 1998, as modified by Wardle et al., 2003b). For each soil sample, 20 individual 10 g (dry weight equivalent, amended to 55% SWHC on a dry weight basis) subsamples were each placed in separate 130-mL airtight containers as described above for basal respiration. Soils in 19 of these containers were each amended with a 1 mL solution of a different substrate, followed by 1 mL of distilled water (bringing the soil moisture content to 90% SWHC). The 20th container was amended with 2 mL of water only. The substrates used are a subset of those used by Degens et al. (1998) and included L-asparagine, L-cysteine, L-glutamic acid, DL-histidine, L-serine, DL-tyrosine, D-glucosamine, L-glutamine, *N*-methyl glucamine, D-gluconic acid, citric acid, malonic acid, quinic acid, urocanic acid, L-ascorbic acid, α-ketobutyric acid, α-ketoglutaric acid, DL-malic acid, and pantothenic acid. Substrates were added to soils at the same mg substrate-C per gram dry soil as those described by Degens and Harris (1997). Following amendment, the containers were incubated and the headspace sampled (after 1 and 4 h of incubation) for CO_2 concentration exactly as described for basal respiration. The respiration value for the sample not amended with substrate was then subtracted from the respiration values of each of the 19 corresponding substrate-amended samples, so as to provide an absolute measure of respiratory response of the microflora to each of the added substrates.

Soil nematodes and enchytraeids were extracted from a 100 g wet weight subsample of fresh soil from each sample, using a tray variant method as described by Yeates (1978). Gravimetric moisture content was determined so that response variables could be expressed on a dry weight basis. Nematodes and enchytraeids were counted live at 40× magnification before fixing the suspension by the addition of an equal volume of boiling 8% formaldehyde. Subsequently an average of 122 nematodes per subsample were identified to nominal genus, and placed into six feeding groups occupying three trophic levels, following Yeates et al. (1993). These trophic groups (with component functional groups in brackets) are: microbe feeders (bacterial feeders and fungal feeders), predators

(top predators and 'omnivores', i.e. predators that feed at more than one trophic level), and herbivores (plant feeders and plant associates).

2.3. Data analysis

To specifically test whether soil resource quality directly impacted on the activity and quantity of soil microbes we focused on the ratio of biomass or populations of organisms to total soil carbon. The Shannon-Weiner diversity index was used as a relative diversity measure, and was determined for each soil sample from the SUP data ('catabolic diversity' of the microbial community; Degens, 1998), PLFA concentration data, and nematode data. Principal components analysis was used to describe differences in community composition among chronosequence stage for each of these sets of data; for the nematode data these analyses were performed at the level of feeding groups. For each response variable (including the diversity indices and ordination score values), analysis of variance (ANOVA) was used to test for effects of treatment (chronosequence stage); differences among means were evaluated using the least significant difference (LSD) test. For all data analysis, variables were transformed (by log- or square root-transformation) as necessary, to satisfy assumptions of parametric analyses such as normality and homogeneity of variances.

3. Results

With increasing chronosequence age, there were significant ($P=0.05$) increases in percent organic C in the organic layer (stage 1, 2, 3, 4 = 11, 37, 38 and 47% C, respectively), presumably as a result of biotic and abiotic disturbance incorporating more mineral soil into organic layer at the earlier phases of the chronosequence (Table 1). Similar increases were observed for organic layer N (which increased from 0.50 to 1.14% throughout the sequence), while significant declines were noted for total P (from 0.085 to 0.021%) and bicarbonate-extractable P (from 0.0024 to 0.0012%). Comparable patterns were also recorded for the mineral soil, with increases throughout the chronosequence of organic C (stage 1, 2, 3, 4 = 5, 17, 20 and 46% C, respectively) and total N (from 0.31 to 1.28%) but declines for total P (from 0.074 to 0.019%) and bicarbonate extractable P (from 0.0016 to 0.0010%). The second soil layer, defined here as the 'mineral soil layer', at stage 4 of the chronosequence is typical of an organic soil O horizon (Milne et al., 1995), however, we have continued to use the term 'mineral soil' to distinguish this layer from the organic layer from the same core. Soil pH declined across the sequence for both depths, i.e. from 4.7 to 3.9 in the organic layer and from 4.8 to 3.9 in the mineral soil (Table 1). The quality of the soil organic matter declined through the sequence; there were usually highly significant increases over time in ratios of C to N, C to P, and N to P in both the organic layer and mineral soil layer (Fig. 1; Table 1). Forms of mineral nitrogen also changed throughout the sequence, with declines over time in both the organic layer and mineral soil layer of nitrate concentration and increases in both layers of ammonium concentration (Fig. 2, Table 1).

Table 1
F- and *P*-values derived from one-way ANOVA testing for effects of chronosequence stage on soil nutrient variables

Variable	Organic layer		Mineral soil	
	F	*P*	*F*	*P*
pH	23.21[a]	<0.001	81.59	<0.001
TC (%)	9.54	0.002	9.29	0.002
TN (%)	6.19	0.009	12.22	0.001
TP (mg kg^{-1})	66.21[a]	<0.001	48.75	<0.001
C to N ratio	34.28	<0.001	12.26	0.001
C to P ratio	83.19[a]	<0.001	30.02	<0.001
N to P ratio	107.65[a]	<0.001	91.61[a]	<0.001
NO_3^- (mg kg^{-1})	16.20[a]	<0.001	8.48	0.003
NH_4^+ (mg kg^{-1})	6.36	0.008	5.58	0.013

TC, total carbon; TN, total nitrogen; TP, total phosphorus.
[a] Variable square root transformed.

When data were presented on a per unit soil weight basis, measurements of BR, SIR and total PLFAs were not significantly related to chronosequence stage (Table 2), while the sum of microbial respiratory responses to all 19 added substrates used in the SUP bioassay (total SUPs) showed significant increases over time for both the organic layer and mineral soil layer (Fig. 3, Table 2). However, when data were expressed on a per unit soil C basis, there were significant declines over time in both soil layers for SIR, total respiratory responses to SUP substrates, total PLFAs, and bacterial PLFAs, though not for fungal PLFA (Table 2, Fig. 3). Further, there were significant increases in the ratio of fungal to bacterial PLFAs for both soil depth layers (Table 2, Fig. 3). Diversity of PLFAs (Shannon-Weiner index) was unrelated to chronosequence stage for the organic layer, but showed a weak increase with stage for the mineral soil layer ($P=0.054$). Meanwhile, microbial catabolic diversity (assessed using the SUP data) was significantly less for stage 4 (Shannon-Weiner index = 3.72) than for the other three stages (Shannon-Weiner indices = 3.87, 3.83 and 3.84 for stages 1, 2 and 3, respectively) in the organic layer, but was unresponsive to chronosequence stage in the mineral soil layer. Ordination analyses showed that microbial community structure was significantly affected by chronosequence stage for both the organic layer and mineral soil layer and for both the PLFA and SUP data sets (Table 2, Fig. 4).

In the organic layer, total nematode densities declined significantly throughout the chronosequence, from 58 individuals per gram soil in stage 1, to 4 individuals per gram soil in stage 4 (Table 3). However, only two of the six trophic groupings of nematodes showed significant

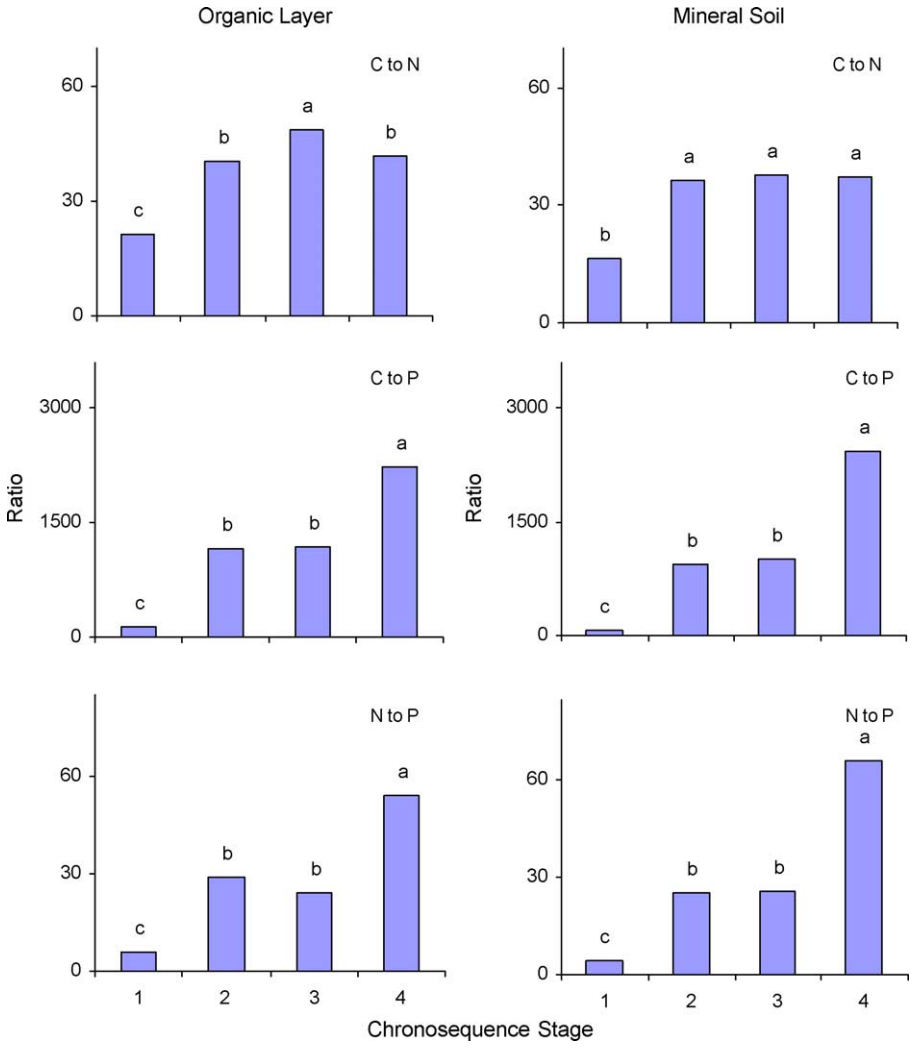

Fig. 1. C:N, C:P, and N:P ratios of organic layer and mineral soil C to N, C to P and N to P in relation to chronosequence stage. Within each panel, bars topped by the same letter are not significantly different at $P=0.05$ (least significant difference test following one-way ANOVA).

declines at $P=0.05$ in the fungal feeding and plant feeding nematodes (Table 3). Total nematode densities were not significantly related to chronosequence stage in the mineral soil layer, although two groups (predatory and omnivorous nematodes) showed a significant decline (Table 3). A total of 60 nematode taxa were differentiated; bacterial-feeding nematodes included *Alaimus*, *Aphanolaimus*, *Cephalobus*, *Chromadoridae*, *Cryptonchus*, *Diplogaster*, *Etamphidelus*, *Euteratocephalus*, *Heterocephalobus*, *Monhystera*, *Pakira*, *Panagrolaimus*, *Plectus*, *Prismatolaimus*, *Rhabditidae*, *Rhabdolaimus*, *Teratocephalus*; fungal-feeding nematodes included *Anguinidae*, *Aphelenchoides*, *Diphtherophora*, *Doryllium*, *Leptonchus*, *Tylencholaimus*; predatory nematodes included *Clarkus*, *Cobbonchus*, *Ethmolaimus*, *Iotonchus*, *Ironus*, *Nygolaimus*, *Seinura*, *Tobrilus*, *Tripyla*; plant-feeding nematodes included *Criconemoides*, *Hemicycliophora*, *Hoplolaimidae*, *Longidorus*, *Paratylenchus*, *Pratylenchus*, *Radopholus*; plant associated nematodes included *Axonchium*, *Belondiridae*, *Campbellenchus*, *Dorylaimellus*, *Falcihasta*, *Tylenchus*; omnivorous nematodes included *Aporcelaimus*, *Chromadoridae*, *Dorylaimus*, *Enoplidae*, *Eudorylaimus*, *Labronema*, *Mesodorylaimus*. Only 20 taxa had populations exceeding 1 per gram in the organic layer, with only *Rhabditidae* 1, *Tylencholaimus* 2, and *Tylenchus* 2 exceeding 10 per gram at any site. Enchytraeid densities were unrelated to chronosequence stage for both soil depth layers (Table 3). When nematode densities were expressed on the basis of numbers per unit soil C (i.e. densities/%C), there were substantial, and statistically significant declines in all six nematode trophic groupings, for both the organic layer and mineral soil layer (Table 3, Figs. 5 and 6). Further, enchytraeid densities per unit soil C declined significantly at $P=0.05$ in the organic layer (from 0.030 individuals/%C in stage 1 to zero individuals detected/%C in stage 4), though not in the mineral soil.

There were some significant nematode community compositional changes across the sequence. The ratio of

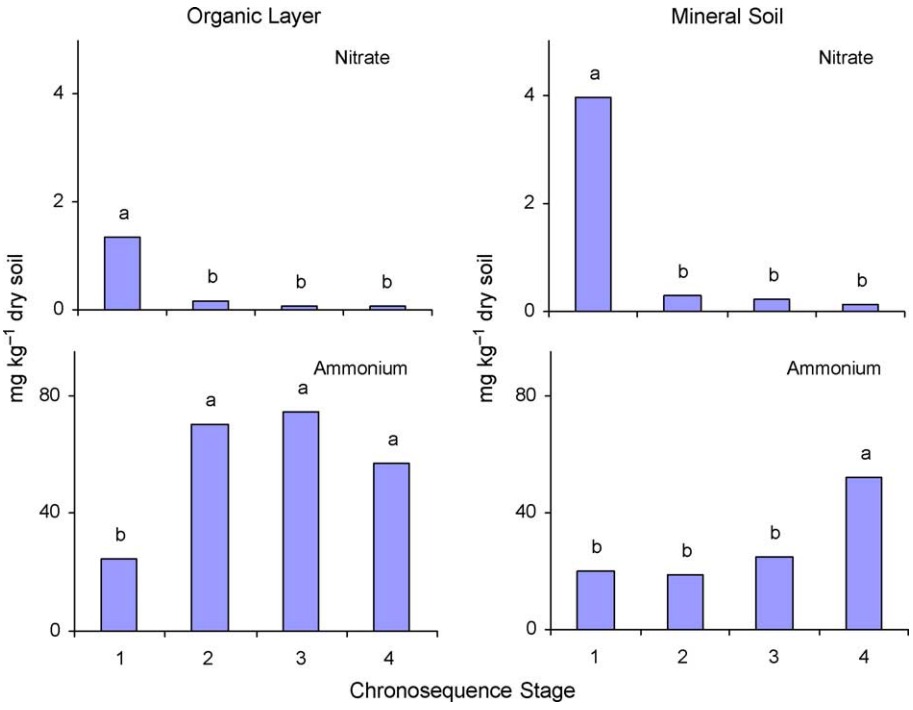

Fig. 2. Organic layer and mineral soil nitrate and ammonium concentrations in relation to chronosequence stage. Within each panel, bars topped by the same letter are not significantly different at $P=0.05$ (least significant difference test following one-way ANOVA).

fungal feeding to bacterial feeding nematodes increased substantially for both soil depth layers, although this was only statistically significant for the organic layer (Table 3, Fig. 7). Further, ordination analyses revealed significant nematode community differences across chronosequence stages when these analyses were performed at the level of functional groups, but only for the organic layer (Table 3, Fig. 8). Nematode diversity, assessed using

Table 2
F- and *P*-values derived from one-way ANOVA testing for effects of chronosequence stage on soil microbial response variables

Variable	Organic layer		Mineral soil	
	F	*P*	*F*	*P*
Basal respiration (μg CO_2-C g^{-1} soil h^{-1})	0.97	0.437	1.41	0.288
Basal respiration to soil C ratio	3.27	0.058	2.27	0.133
SIR (μg CO_2-C g^{-1} soil h^{-1})	2.13	0.149	1.21	0.381
SIR to soil C ratio	10.91	0.001	7.20	0.005
Total PLFA (relative nmoles g^{-1} soil)	1.92	0.227	0.61	0.635
Total PLFA to soil C ratio	26.55	0.001	8.77	0.019
Fungal PLFA (relative nmoles g^{-1} soil)	3.28	0.100	2.97	0.136
Fungal PLFA to soil C ratio	1.66	0.273	2.16	0.212
Bacterial PLFA (relative nmoles g^{-1} soil)	1.47	0.313	5.40	0.050
Bacterial PLFA to soil C ratio	31.97	<0.001	14.41	0.007
Fungal to bacterial PLFA ratio	9.93	0.009	5.69	0.046
Total SUP (μg CO_2-C g^{-1} soil h^{-1})	8.35	0.003	3.61	0.046
Total SUP to soil C ratio	13.33	<0.001	12.79	0.001
Shannon-Weiner diversity index PLFA	2.77	0.133	5.17	0.054
Shannon-Weiner diversity index SUP	4.09	0.032	2.02	0.164
PCI (PLFA data)	14.41	0.004	3.45	0.108
PCII (PLFA data)	1.15	0.402	12.62	0.009
PCI (SUP data)	6.50	0.007	5.21	0.016
PCII (SUP data)	3.05	0.069	1.11	0.382

SIR, substrate induced respiration; PLFA, phospholipid fatty acid; total SUP, total sum of the respiratory responses of soil to all 19 added substrates; PCI and PCII are primary and secondary ordination scores derived from principal component analysis.

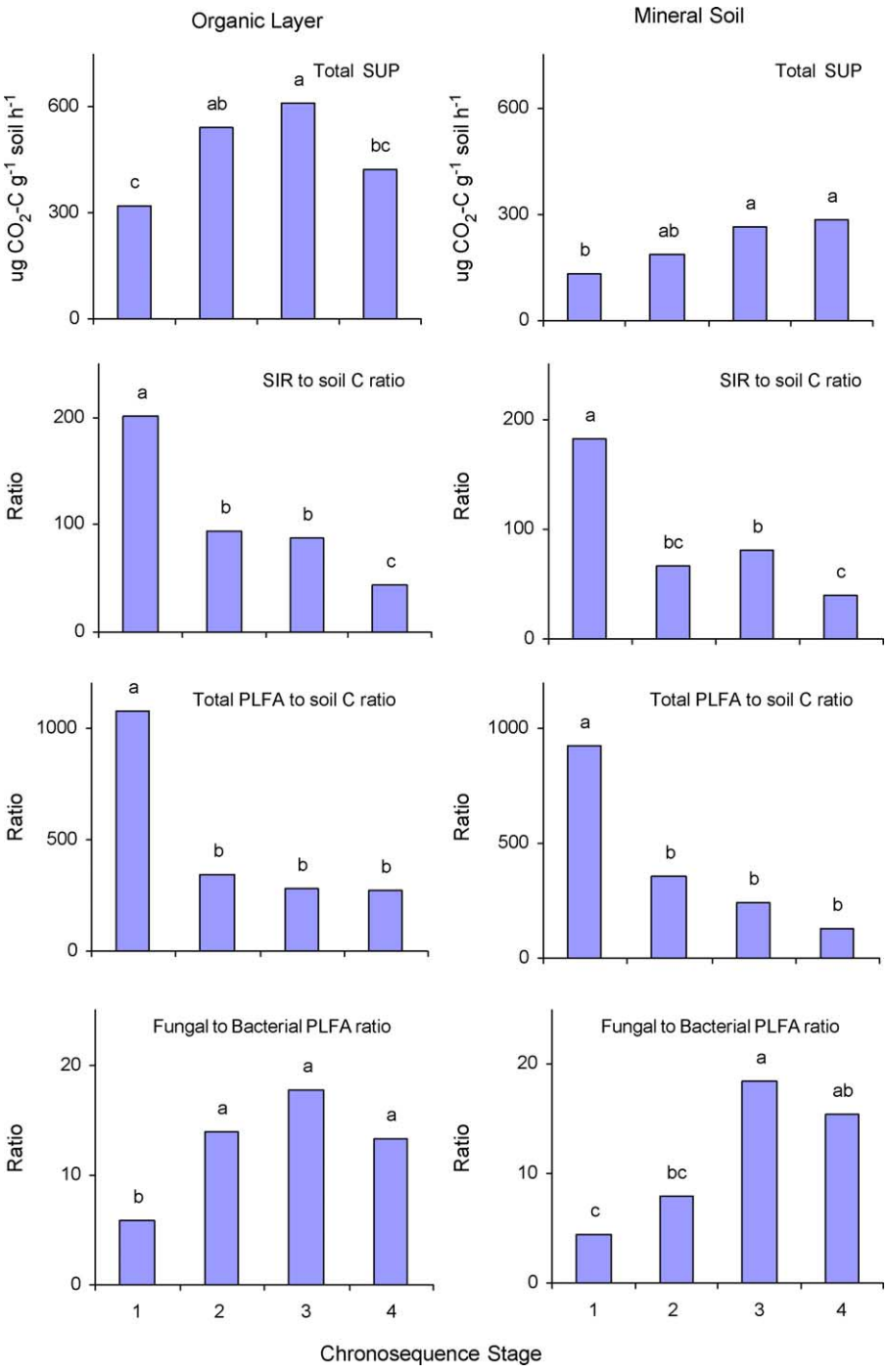

Fig. 3. Soil microbial variables in relation to chronosequence stage in organic layer and mineral soil. Within each panel, bars topped by the same letter are not significantly different at $P=0.05$ (least significant difference test following one-way ANOVA).

the Shannon-Weiner index, was unrelated to chronosequence stage for both soil depths (Table 3).

4. Discussion

Our study shows substantial increases in carbon to nutrient ratios in both the organic layer and mineral soil layer during long-term ecosystem decline, and this is consistent with investigations of other long-term chronosequences that have shown increasing limitations by major nutrients over time (Chapin et al., 1994; Crews et al., 1995; Wardle et al., 1997). It is likely that this ecosystem decline is caused in part by increasing limitation of phosphorous over time, since as ecosystem decline progressed, the organic layer and mineral soil N to P ratio increased

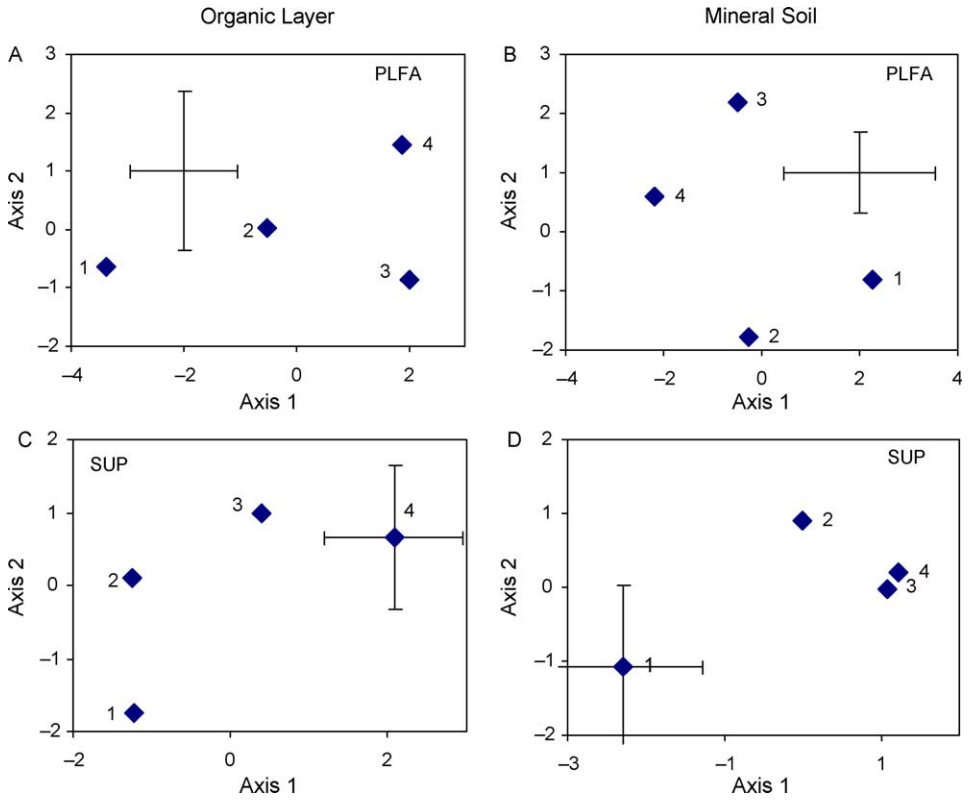

Fig. 4. Principal component analysis ordination plots of microbial community structure in organic layer and mineral soil, assessed using phospholipid fatty acid (PLFA) data or substrate utilisation profile (SUP) data. For each panel, the numbers beside each point relates to the chronosequence stage. The horizontal and vertical bars relate to least significant difference at $P=0.05$ for axes 1 and 2, respectively. The first axis explained 38, 40, 20 and 22% of total variation for panels A–D, respectively. The second axis explained 19, 23, 18, 14% of total variation for panels A–D, respectively.

Table 3
F- and *P*-values derived from one-way ANOVA testing for effects of chronosequence stage on soil nematode and enchytraeid data per gram dry soil

Variables	Organic layer		Mineral soil	
	F	*P*	*F*	*P*
Total nematodes	3.75	0.041	1.74[a]	0.212
Total nematodes to soil C ratio	43.36[a]	<0.001	11.56[a]	0.001
Total enchytraeids	3.26	0.059	1.93	0.178
Total enchytraeids to soil C ratio	4.68[a]	0.022	3.15[a]	0.065
Bacterial-feeding nematodes	2.94	0.076	2.98[a]	0.074
Fungal-feeding nematodes	5.32	0.015	1.21[a]	0.349
Predatory nematodes	2.97	0.074	4.31[a]	0.032
Plant-feeding nematodes	4.12	0.032	1.70[a]	0.220
Plant-associated nematodes	3.22	0.061	0.92[a]	0.459
Omnivorous nematodes	2.79	0.086	4.46[a]	0.025
Fungal-feeding to bacterial-feeding nematodes	13.74	<0.001	1.77[a]	0.207
Bacterial-feeding nematodes to soil C ratio	53.74	<0.001	11.29	0.001
Fungal-feeding nematodes to soil C ratio	26.91	<0.001	7.53	0.004
Predatory nematodes to soil C ratio	21.45	<0.001	18.29	<0.001
Plant-feeding nematodes to soil C ratio	10.97	0.001	14.16	<0.001
Plant-associated nematodes to soil C ratio	31.68	<0.001	8.80	0.002
Omnivorous nematodes to soil C ratio	27.63	<0.001	16.89	<0.001
Shannon-Weiner diversity index for nematode genera	1.58	0.246	1.70	0.220
PCI (nematode data to genus level)	1.41	0.288	0.83	0.501
PCII (nematode data to genus level)	1.22	0.345	0.20	0.893
PCI (nematode data to feeding group level)	3.82	0.039	2.74	0.089
PCII (nematode data to feeding group level)	3.81	0.039	1.87	0.188

PCI and PCII are primary and secondary ordination scores derived from principal component analysis.
[a] Variable Log_2 transformed.

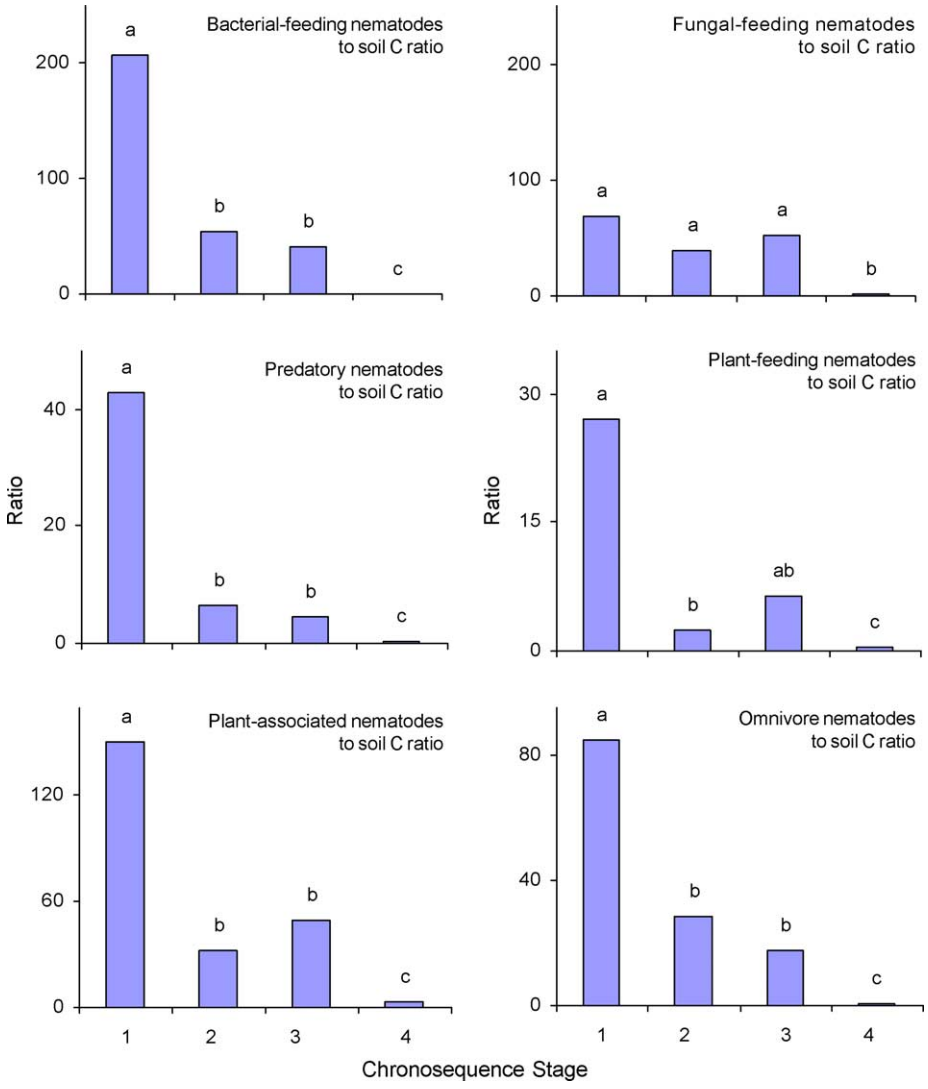

Fig. 5. Densities of nematodes in each of six feeding groups for the organic layer (expressed on a per unit soil C basis), in relation to chronosequence stage. Within each panel, bars topped by the same letter are not significantly different at $P=0.05$ (least significant difference test following one-way ANOVA).

significantly beyond the Redfield Ratio (16:1), or the ratio beyond which P becomes increasingly limiting to living organisms relative to N (Redfield, 1958; Sterner and Elser, 2002). This trend of increasing P limitation relative to that of N is consistent with what has been found for two other long-term chronosequences, i.e. the Franz Josef Glacier chronosequence (Walker and Syers, 1976; Richardson et al., 2004) and the Hawaiian volcanoes chronosequence (Crews et al., 1995; Hedin et al., 2003).

Measurements of soil microbial biomass, expressed on a per unit soil weight basis, did not decline throughout the chronosequence. Instead, when the microbial response to all substrates used for the SUP bioassay were summed for each soil sample (total SUPs), there was a significant increase over time. The likely reason is that as the total organic C concentration increased in the soils with increasing chronosequence stage, this resulted in a greater net amount of resources being present to support the microbial biomass. The lower C concentration in soils from stage 1 of the sequence is likely to be due to greater vertical soil mixing resulting from soil macrofaunal activities (causing mineral soil deeper in the soil profile with low C status to be brought closer to the soil surface) and regular input of mineral soil particles to the soil surface through alluvial deposition. However, when microbial biomass variables were calculated on a per unit soil C basis, there were substantial declines of biomass with increasing chronosequence stage. An expression of microbial variables as a ratio of soil C is relevant for interpreting the impact that aboveground changes in vegetation have on the belowground subsystem, because the central premise being assessed is that during ecosystem decline the quality of the organic matter entering the soil is of a lower quality for microbial metabolism and hence cannot support the same level of organisms as can litter produced during earlier chronological stages. This is attributable to diminishing quality of soil carbon substrates

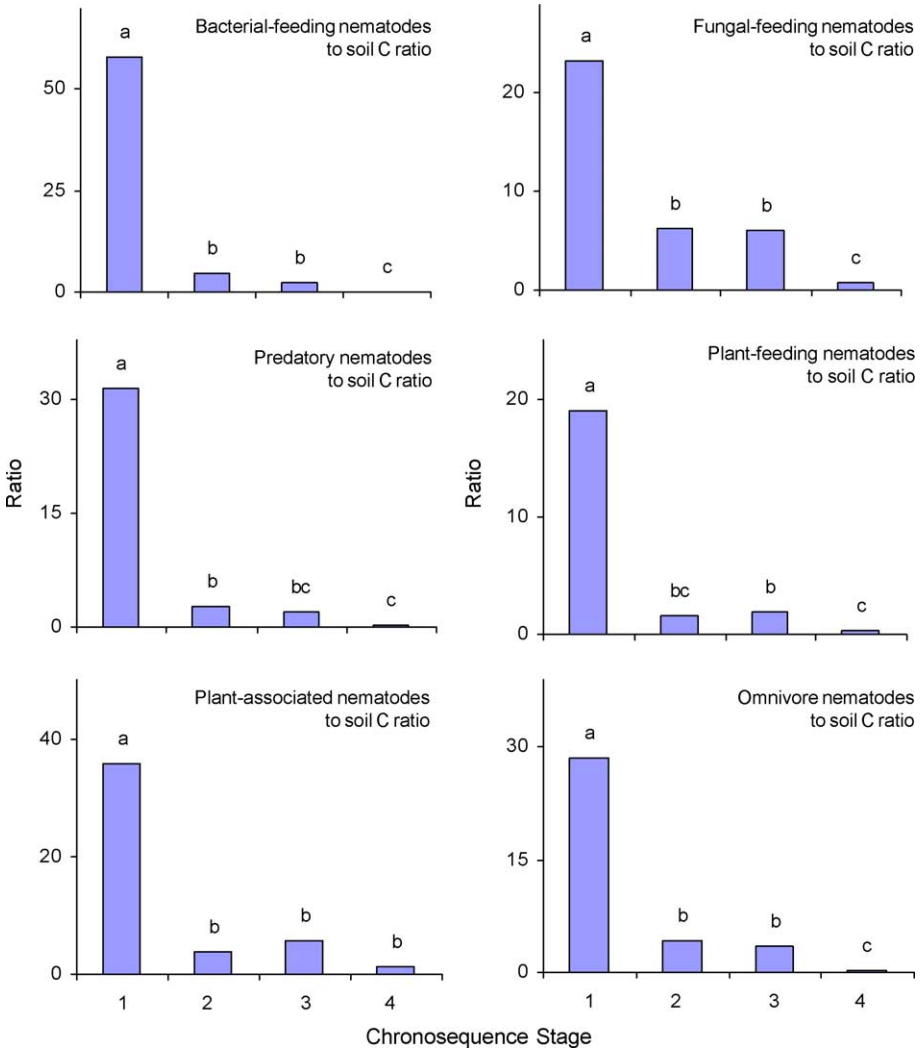

Fig. 6. Densities of nematodes in each of six feeding groups for the mineral soil layer (expressed on a per unit soil C basis), in relation to chronosequence stage. Within each panel, bars topped by the same letter are not significantly different at $P=0.05$ (least significant difference test following one-way ANOVA).

being able to support microbial metabolism during ecosystem decline (see Insam and Domsch, 1988; Wardle, 1992), and is reflective of increased ratios of soil C to N, C to P and N to P across the sequence. These results are consistent with those of other chronosequence studies that have shown declines in soil microbial activity (Wardle and Ghani, 1995), plant litter decomposition rates (Crews et al., 1995), and supply rates from the soil of plant available nutrients

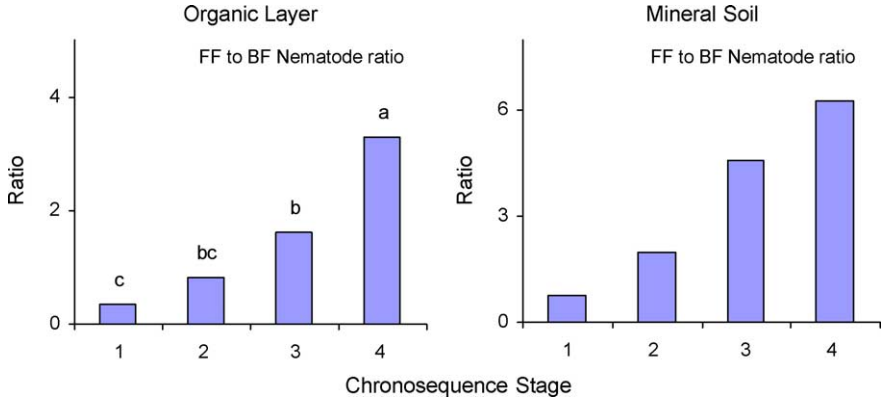

Fig. 7. Ratios of fungal feeding (FF) to bacterial feeding (BF) nematodes in organic layer and mineral soil, in relation to chronosequence stage. Within each panel, bars topped by the same letter are not significantly different at $P=0.05$ (least significant difference test following one-way ANOVA).

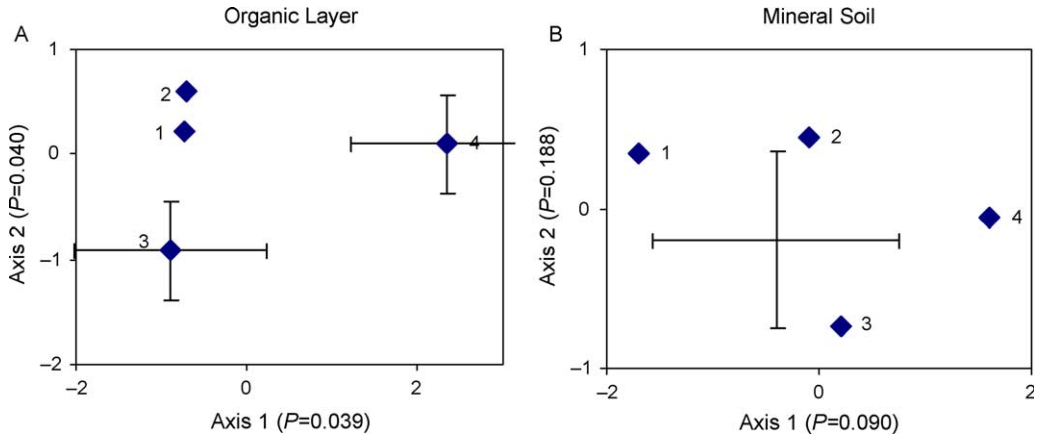

Fig. 8. Principal component analysis ordination plots of nematode community structure at the level of nematode feeding groups in organic layer and mineral soil. For each panel, the numbers beside each point relates to the chronosequence stage. The horizontal and vertical bars relate to least significant difference at $P=0.05$ for axes 1 and 2, respectively. The first axis explained 67 and 61% for panels A and B, respectively. The second axis explained 15 and 14% for panels A and B, respectively.

(Chapin et al., 1994), during the decline phase of ecosystem succession.

Declining resource quality during ecosystem decline had adverse multitrophic effects on the soil nematode and enchytraeid fauna. For some trophic groups this was apparent when results were expressed on a density per unit soil weight basis, but for all trophic groups there were very strong trends when results were expressed on a per unit soil C basis. The effects were consistent for all three trophic levels, indicating that the effects of reduced soil organic matter quality on the soil microflora had effects that propagated through at least two higher trophic levels, i.e. microbe-feeders (bacterial- and fungal-feeding nematodes, and enchytraeids), and predators (i.e. top predatory and omnivorous nematodes). The fact that the three consumer trophic levels (microbes, microbe feeders and predators) responded in the same way to the underlying resource quality gradient across the chronosequence is consistent with the study of Mikola and Setälä (1998), which found these three trophic levels to respond in the same way to increased resource availability in experimental soil microcosms. However, this pattern is inconsistent with the study of Wardle and Yeates (1993) on microbial-nematode soil food webs, and some trophic dynamic theories (Hairston et al., 1960; Oksanen et al., 1981); these other studies indicated that only some trophic levels of food chains should show strong positive responses to increased resource availability because other trophic levels are governed by top down (predator) regulation.

There were significant unidirectional changes across the chronosequence for both the microbial community (assessed using both PLFA and SUP data) and the nematode community. This did not, however, translate to strong shifts in either microbial or nematode diversity. The most significant compositional changes that we observed were with regard to the relative importance of the bacterial-based to fungal-based energy channels of the soil food web. Across the chronosequence, there were substantial increases in both the fungal to bacterial ratio (PLFA data) and the fungal feeding to bacterial feeding nematode ratio. This indicates that as the decline phase of succession proceeds and nutrients become more limiting, fungal metabolism become increasingly favoured relative to bacterial metabolism, and this shift results in changes in higher trophic levels. Because fungal biomass turns over more slowly than that of bacteria, and because the proportion of fungal biomass that is consumed by soil animals is lower than that for bacterial biomass, fungal-dominated food webs are far more effective at conserving nutrients (Coleman et al., 1983; Moore and Hunt, 1988; Wardle and Yeates, 1993). We would expect the increasing role of the fungal-based energy channel with increasing chronosequence stage to contribute to greater retention and lower rates of mineralisation of soil nutrients.

There is recognition that during the build-up phase of long-term chronosequences there is an increase in the quantity of available soil nutrients, soil biota, and rates of aboveground and belowground ecosystem processes. Our data show that during the chronosequence decline phase (during which the stature and biomass of vegetation diminishes), there are significant reductions in the levels of soil organisms, on a per unit soil C basis, responsible for carrying out key soil processes, notably decomposition and the release of plant-available nutrients from soil organic matter. During ecosystem decline, the quality of organic matter entering the soil subsystem is expected to decline. Our results indicate the potential for important feedbacks between the aboveground and belowground subsystems, in which the quality of soil organic matter declines to the extent that it cannot sustain the former levels of decomposer food web organisms during ecosystem decline. It is likely

that this in turn results in feedbacks that have the potential to culminate in reduced plant nutrient acquisition and ultimately reduce ecosystem productivity, thus further promoting ecosystem decline.

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