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Ecosystem service and biodiversity trade-offs in two woody successions

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Summary

1. Many grasslands worldwide are undergoing succession to woody vegetation, causing complex effects on carbon (C) sequestration, nutrient cycling and biodiversity. Land managers are frequently tasked with maximizing ecosystem services and biodiversity. Nonetheless, there are few studies quantifying trade-offs between ecosystem services and biodiversity during early woody succession.

2. We assessed the consequences of woody succession for C stocks, above- and below-ground taxa richness (plants, nematodes, mites, microbes, fungi), and soil ecosystem function at one site with a native tree, *Kunzea ericoides*, and one site with a non-native tree, *Pinus nigra*, both establishing in conservation grasslands.

3. Woody succession at both sites was associated with large gains in above-ground C stocks and, under *P. nigra*, losses from the mineral soil-C pool.

4. Taxa richness responses were complex, nonlinear and incongruent. While some taxa showed initial increases in richness with woody succession (e.g. plants), other taxa had rapid declines (e.g. plant-feeding and plant-associated nematodes, oribatid mites).

5. Below-ground ecosystem functioning shifted towards increased bacterial energy channels with woody succession, despite no change in bacterial or fungal biomass or fungal hyphal lengths. Most other soil measures were consistent with literature expectations (increased C:N ratios, release of recalcitrant phosphorus).

6. *Synthesis and applications*. Our gradient-based measurements of woody succession effects on ecosystems did not follow expectations based on comparing end-points of grasslands to homogeneous mature forest. The discordance of biodiversity responses across taxonomic groups suggests that managers cannot rely on the indicator-species concept to ensure conservation of cryptic biodiversity. Carbon sequestration and biodiversity followed non-congruent patterns, with significant losses of taxa richness from some functional groups during woody succession. Management to maximize individual ecosystem services such as carbon sequestration may therefore result in significant negative effects on biodiversity of some, but not all, taxa.

Key-words: below-ground processes, biological invasion, carbon sequestration, ecosystem properties, grassland, nutrient cycling, soil biota

Introduction

Many grasslands are undergoing succession to woody vegetation due to land use change, exotic woody invasion, fire suppression and intentional management (Hibbard *et al.* 2001; Richardson & Rejmánek 2004). Increasing woodiness may result in increases in above-ground carbon (C) sequestration (Hughes *et al.* 2006; Liao *et al.* 2008), but be negatively correlated with other ecosystem services and biodiversity. This may include, for example declines in plant diversity (Báez & Collins 2008) and native-beetle species richness (Pawson *et al.* 2010)

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following woody invasion. Nonetheless, land managers are frequently tasked with simultaneously maximizing both ecosystem services and biodiversity or to defend decisions to emphasize one over the other (Rodriguez *et al.* 2006; Pejchar & Mooney 2009). For example, a NZD$10 million project to preserve biodiversity by removing non-native invasive pines from New Zealand grasslands was recently put on hold because of a NZD$3 million liability for C-loss under the Kyoto Protocol (Williams 2009).

One difficulty in assessing ecosystem service trade-offs is that humans have an above-ground worldview, yet most ecosystem services are soils based (van der Putten *et al.* 2004). Globally, soils contain an estimated 2500 Gt of C; about three-fold greater than the atmospheric pool, and four-fold greater than the biotic pool (Lal 2004). The soil-C pool can be susceptible to woody invasion, with average losses of 12–15% of soil-C following pine establishment (Guo & Gifford 2002). Soils also contain the greatest pool of terrestrial biological diversity typically exceeding associated plant diversity by one to three orders of magnitude (Roesch *et al.* 2007). These highly diverse communities can be directly affected by increasing woodiness, although shifts in composition may be more common than declines in species richness (Dickie *et al.* 2009; Macdonald *et al.* 2009). Finally, soils are the basis of nitrogen (N) fixation, phosphorus (P) cycling and water storage (van der Putten *et al.* 2004), which may be altered by increasing woodiness (Berthrong, Jobbagy & Jackson 2009; Pejchar & Mooney 2009). Despite the links between soils and ecosystem services, few below-ground studies have simultaneously quantified multiple-ecosystem services and biodiversity across gradients of land-cover change.

Here, we report the results of two parallel studies of woody succession in grasslands, assessing above- and below-ground aspects of ecosystem change. We apply the same approach and methods to two different sites where ectomycorrhizal trees, one native and one non-native, are establishing as monodominant forests in abandoned agricultural grazing lands. These represent two common landscape trajectories of conservation land in New Zealand and are similar to ectomycorrhizal woody successions in grasslands worldwide (e.g. *Pinus*, *Quercus*, *Betula*, *Alnus*, *Eucalyptus*, *Salix)*. Our objectives were to understand the effects of succession on a range of ecosystem processes that underpin ecosystem services, including C-pools and fluxes and soil nutrient dynamics, and to investigate trade-offs between ecosystem services and above- and below-ground biodiversity. We expected that during the course of succession from arbuscular mycorrhizal grasslands to ectomycorrhizal woody vegetation there would be: (i) a large increase in total C storage associated with woody biomass (Hughes *et al.* 2006; Liao *et al.* 2008); (ii) a concomitant loss of biodiversity, particularly of non-woody plants due to competition from trees, but also from other taxa as plant communities become less structurally complex (Báez & Collins 2008; Pawson *et al.* 2010); (iii) changes in soil nutrient cycling, due to the effects of recalcitrant tree litter and tree-associated ectomycorrhizal fungi, which have greater abilities to utilize organic nutrient pools than arbuscular mycorrhizal fungi (Binkley & Giardina 1998; Read, Leake & Perez-Moreno 2004; Lambers *et al.* 2008); and (iv) a shift to greater fungal dominance of soil ecosystems, as greater C inputs to soil and other effects of trees on soil favour fungi over bacteria (Harris 2009; Bardgett & Wardle 2010).

Our design was not intended to directly compare native and non-native succession, but where commonalities occur they may indicate species-independent patterns. This research has three points of difference from prior studies on the effects of grassland afforestation (Chapela *et al.* 2001; Berthrong, Jobbagy & Jackson 2009; Macdonald *et al.* 2009; Pawson *et al.* 2010). First, we studied self-established trees, thereby avoiding planting or management effects. Secondly, we measured a full range of natural tree densities, rather than focusing solely on the grassland vs. forest end-points. In natural successional processes, spatial heterogeneity is common and can result in non-linear responses of ecosystem processes to tree density. By measuring a full density gradient (0 to >90% tree cover), we attempted to capture this local-scale (20 × 20 m plot) variability. Finally, our simultaneous assessment of C, biodiversity, nutrient pools and soil communities provides a representative cross-section of many aspects of ecosystem processes underpinning ecosystem services. This research also complements recent reviews that have addressed larger scale measurement of services during woody succession (e.g. Pejchar & Mooney 2009).

Materials and methods

We established 15 research plots (20 × 20 m) spanning a gradient from no trees (0% cover) to near 100% tree cover (Fig. 1, Tables S1

Fig. 1. Woody succession to *Pinus nigra* at Mt. Barker (top) and *Kunzea ericoides* at Avoca (bottom).

Supporting Information). The first site, Mt. Barker, is a *Pinus nigra* Arnold non-native invasive succession in previously grazed grassland on a river terrace in Mid-Canterbury, New Zealand, with the largest trees 22 years old and up to 14 m in height. The second site, Avoca, is a *Kunzea ericoides* (A. Rich.) Joy Thomps. native succession in previously grazed grassland on a river terrace in the Korowai/Torlesse Tussocklands Park, with the largest trees measured at 54 years old and up to 8 m in height. *Kunzea* is a small-leafed, light-demanding, evergreen, ectomycorrhizal Myrtaceous tree that forms dense stands in early succession. The two sites are about 21 km apart; geographical coordinates, soils, vegetation and microclimate data are in Tables S1 and S2 in Supporting Information. To find plot centres in a stratified random fashion, we ran transects crossing areas of high and low density of trees, placing potential plot centres at predetermined regular intervals. Preliminary density estimates were made, and plots selected from potential plot centre locations in order to span the tree-cover gradient, with three plots at each end of the gradient (0% and >90%).

ABOVE-GROUND CARBON POOLS

Above-ground C pools were estimated based on tree and shrub biomass derived from allometric equations and direct measurement of diameters and heights of trees and volumes of shrubs (details in Dickie *et al.* 2009; Landcare Research Report LC0809/161, Lincoln, New Zealand). All vascular plant species rooted within plots were identified by species. We measured groundcover biomass by harvesting all vegetation in two 0·25-m^2 samples within each plot, sorting into grass, small-woody and herbaceous biomass, drying (60 °C) and weighing. We conservatively assumed that plant C was 45% of dried biomass (Schlesinger 1991).

SOIL SAMPLES

Within each plot we took eight 65-mm-diameter soil cores of the A-horizon (*c.* 100 mm depth at both sites). Six cores (two central and four at equidistant points 2·5 m from the centre of plot) were bulked for chemical, DNA, phospholipid fatty acid (PLFA), and microbial catabolic response profiles (CRP). Two cores were taken to quantify root biomass. Where the litter layer was >2·5 mm in average depth, six 100-mm diameter samples of the total litter depth were collected: four litter samples for microarthropod extraction, and two litter samples for nematode extraction and chemical analysis. All samples were stored at 4 °C until processing. Bulk density was measured on three replicate 62-mm diameter, 30-mm long sample rings on three forest and three grassland (no tree) plots at each site. Mean bulk density was used for analyses as neither site showed a significant response of soil bulk density to tree succession ($P > 0{\cdot}5$ for both sites).

SOIL RESPIRATION

We made four seasonally representative soil respiration measurements (PPSystems International, Herts, UK) at each site. Four permanent collars were installed to a depth of 100 mm in each plot 3 months prior to starting measurements. Dry litter was removed from the collar before taking measurements of soil respiration and temperature (50 mm depth), and then replaced. We assumed that the basal respiration rate at 10 °C (R_{10}) was seasonally representative and combined the effects of phenology, activity and soil moisture. The R_{10} for each collar was calculated following Lloyd & Taylor (1994) and averaged for each plot. Soil temperature at 50 mm was continuously measured (Hobo Pro v2; Onset Computer Corp., Bourne, MA, USA) in both an open grass and a closed forest plot. Missing data were reconstructed using a nearby permanent weather station (Craigieburn Forest, CF). Daily rainfall was modelled from a combination of storage rain gauges and CF data. A continuous function (piecewise-cubic Hermite interpolation) was fitted to the R_{10} data using Matlab (MathWorks Inc., Natick, MA, USA) to produce half-hourly R_{10} values from each plot, for the whole year. Half-hourly soil temperatures for each plot were modelled from measurement of grass and forest endpoints and tree biomass. Annual soil respiration flux for each individual plot was then calculated using the modelled half-hourly soil temperature at 50 mm and R_{10} values.

ROOTS

Roots were removed from soil using an automatic washer (20 min) followed by hand-sorting. We scanned a subsample of roots using WinRhizo Pro (Regent Instruments Inc., Ottawa, Canada) to determine root length, diameter and volume and visually assessed a second subsample under magnification using grid-line intercept methods to determine the proportion of ectomycorrhizal (*Pinus* or *Kunzea*) and non-ectomycorrhizal roots. All subsamples were dried (60 °C) and weighed to extrapolate subsample values back to the original soil volume sampled. For C calculations, we estimated total root biomass based on root:shoot ratios of 2:1 for grass, 3:1 for forbs, 0·3:1 for small woody vegetation, 0·17:1 for *Kunzea* and 0·2:1 for pine (Hunt *et al.* 2004, A. Watson, pers. comm.) in addition to direct measurement of root biomass in the A-horizon. In grassland plots at Mt. Barker, measured root biomass in the A-horizon exceeded the estimated total root biomass for the entire soil column (mean 0·8 kg m^{-2} measured vs. 0·73 kg m^{-2} estimated), while in forest plots, measured root biomass was 25% of estimated. We therefore used the maximum of estimated or 120% of measured roots (assuming 80% of grassland roots occur in the A-horizon).

BACTERIAL AND FUNGAL COMMUNITIES

Phospholipid fatty acids were measured as described in Bardgett, Hobbs & Frostegård (1996), using gas chromatography with a subset of samples run through a GC-Mass spectrometer to corroborate biomarkers, using 18:2ω9,12 to represent fungi, 16:1ω5 for AMF and cy-17:0, cy-19:0, i-15:0, a-15:0, i-16:0, i-17:0 and 16:1ω7 for bacteria. CRPs were assessed by measuring respiration responses to 17 substrates: amino acids: glutamine, arginine, histidine, L-serine; amines: glucosamine, glutamic acid; simple sugars: glucose, mannose; aromatics: urocanic acid; carboxylic acids: malic acid, gluconic acid, malonic acid, oxalic acid, quinic acid, ascorbic acid, α-ketoglutaric acid and ketobutyric acid (Degens & Harris 1997). We added amino acids, amines, and aromatics at a concentration of 0·3 mol L^{-1}, carbohydrates at a concentration of 1·5 mol L^{-1}, and carboxylic acids at a concentration of 2·0 mol L^{-1}. One millilitre of each substrate was added to 10 g dry weight (60 °C) equivalent of each soil followed by adjusting final moisture content to 75% of water-holding capacity.

Soil DNA was extracted from bulked soil samples using Soil DNA kits (MoBio Laboratories Inc., Carlsbad, CA, USA). Fungal DNA was amplified from soil DNA using ITS1F-FAM and ITS4-VIC primers (Dickie & FitzJohn 2007). Bacterial DNA was amplified using 8F-FAM and 1492r-VIC primers (Fierer & Jackson 2006). We cleaned positive PCR products (ZR-96 DNA Kits; Zymo Research, Orange, CA, USA), digested with *HpyCH4IV* (NEB; New England Biolab, Ipswich, MA, USA) and *Bsu*RI (Fermentas, Burlington, Canada) restriction enzymes for fungi, and *Hha*I (NEB) and *Bsu*RI

for bacteria, denatured with highly de-ionized formamide, and ran samples through capillary electrophoresis with MapMaker 1000 standard (Bioventures Inc., Murfreesboro, TN, USA), using total peak numbers as a measure of richness. *Fungal hyphal lengths* were measured through direct extraction and microscopic (200×) grid-line intercept counting following standard methods (Rillig, Maestre & Lamit 2003). We separated arbuscular mycorrhizal (AM) hyphae in length measurements based on distinct morphology, but saprotrophic and ectomycorrhizal hyphae cannot be distinguished.

FAUNAL SAMPLES

Nematodes were extracted and enumerated using the tray method (Whitehead & Hemming 1965) from a *c*. 100 g wet weight subsample of A-horizon soil and from litter (where present). Approximately 100 individual nematodes from each sample were identified to nominal genus and assigned to functional guilds following Yeates *et al.* (1993). Dominance of bacterial vs. fungal energy channels was measured as the nematode channel ratio (NCR): NCR = B/(B + F), where B and F are the abundances of bacterial- and fungal-feeding nematodes.

Microarthropods were extracted and identified as either Collembola, oribatid mites (to morphospecies) or other mites (to family) from a separate *c*. 100 g wet weight subsample of A-horizon and from litter using the most efficient extraction methods for each substrate type (heptane flotation and Tullgren funnels, respectively; following methods in St. John, Crossley & Coleman, in press). Only A-horizon soil samples were used in analysis of nematodes and mites, as litter was present only on a limited number of plots; however, litter communities were considered in interpretation.

SOIL NUTRIENTS

We moist-sieved (4 mm) remaining A-horizon soil and analysed for total-P (ignition at 550 °C and dissolution in 0·5 M sulphuric acid), inorganic-P (dissolution in 0·5 M sulphuric acid) and organic-P (difference between total and inorganic pools), total C and N (FP2000 C analyser; LECO Corp., St. Joseph, MI, USA), nitrate-N and ammonium-N (10 g soil extracted with 100 mL 2 M KCl and analysed colorimetrically on a QuikChem 8000; Lachat Instruments, Milwaukee, WI, USA), and pH (1:2·5 soil:water; Blakemore, Searle & Daly 1987). We assessed the form of soil phosphorus by sequential extraction of 1 g finely ground (< 150 μm) air-dried soil with 1 M NH_4Cl (discarded), 0·5 M $NaHCO_3$ pH 8·5, 0·1 M NaOH, 1 M HCl and 0·1 M NaOH. We determined the inorganic-P (P_i) concentration in extracts after acid precipitation of organic matter, while total-P in each extract was determined by persulphate oxidation, and the concentration of organic-P (P_o) in each extract by the difference between total P and P_i (methods and references in Scott & Condron 2003).

STATISTICS

We analysed the two sites independently. We tested soil parameter responses to tree succession using linear regression against tree biomass. For each response variable (y) we tested linear ($y \sim$ tree biomass), loglinear [$y \sim \ln$(tree biomass + 1)], and quadratic ($y \sim$ tree biomass + tree biomass^2) models, selecting the model with the lowest AIC (Akaike Information Criterion) value. Responses were considered significant at $P < 0{\cdot}05$, but marginally significant ($0{\cdot}1 > P > 0{\cdot}05$) results are reported where other variables or a similar response across sites supports the result. We used taxonomic richness as a measure of diversity wherever possible, but Shannon diversity (H′) was used for CRP and PLFA measures of microbial diversity, as all catabolic response substrates were utilized and all PLFA markers were found in all samples. Changes in forms of soil P were tested using a MANOVA to ensure overall significance, followed by ANOVAS on individual measurements. All statistics were performed in R (version 2.10.0; R Development Core Team, 2010).

Results

CARBON

Both sites showed linear increases in total C pools (sum of all vegetation, roots, litter and humus and A-horizon) with tree succession (Fig. 2a,b; Table 1). Maximum tree biomass of *Pinus* was more than twice the maximum biomass of *Kunzea*, despite being less than half the age (22 vs. 54 years) and having similar percentage cover (100% and 90%, respectively). The > 10 kg m^{-2} increase in total C with tree succession occurred despite significant rapid, but quantitatively-small, losses of C from grass biomass at both sites, and significant declines in soil-C and other vegetation biomass-C under *Pinus* at Mt. Barker (Table S2 in Supporting Information). The litter and humus-C pool increased under *Pinus* but not *Kunzea*, but was quantitatively small at both sites (Table S2). Total C pools in the highest density tree plots ($n = 3$ per site) compared with grasslands ($n = 3$ per site) show a net increase of 0·47 ± 0·06 kg C m^{-2} a^{-1} (mean ± SE) at Mt. Barker after 22 years of *Pinus* succession, and 0·08 ± 0·02 kg C m^{-2} a^{-1} at Avoca after 54 years of *Kunzea* succession.

Annual soil respiration at Mt. Barker was lower in grassland plots (no trees) than in all other plots (Fig. S1; 547 ± 45 vs. 779 ± 31 g C m^{-2} a^{-1}, respectively), reflecting a correlation of soil respiration with *Pinus* leaf-area index (LAI; loglinear, $P = 0{\cdot}035$, $R^2 = 0{\cdot}48$), which was maximized at intermediate *Pinus* biomass (Fig. S1). There was no effect of *Kunzea* biomass or LAI on annual soil respiration ($P > 0{\cdot}2$).

SPECIES RICHNESS

Species richness of all plants showed unimodal responses to tree biomass at both sites (Fig. 2c,d; Table 1; species richness includes *Pinus* and *Kunzea*). At Mt. Barker this reflected moderate increases in plant-species richness at low *Pinus* densities followed by declines, while at Avoca there were much larger increases in plant richness with *Kunzea* biomass, with little evidence of declines in plant-species richness at high biomass. The proportion of plant species that were non-native increased with *Pinus* biomass at Mt. Barker (loglinear, from 28% to 35% of species, $P < 0{\cdot}001$) but showed no change with *Kunzea* biomass at Avoca (remaining constant at 35% of species). Most plant functional groups and native and non-native plant-species showed similar responses as total plant richness (Table S2).

In contrast, taxa richness of both plant-feeding and plant-associated nematodes in the A-horizon declined rapidly with both *Pinus* and *Kunzea* (Fig. 2c,d; Table 1; groups separately in Table S2). At Mt. Barker taxa richness of fungal-feeding,

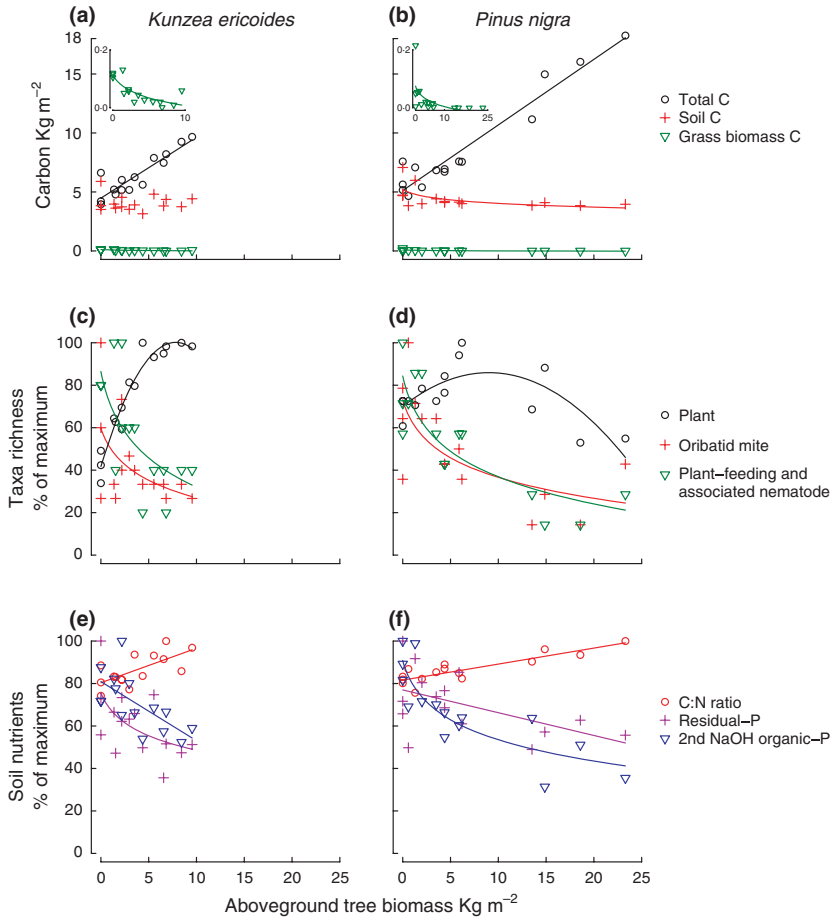

Fig. 2. Changes in C pools (a, b; total C is sum of all vegetation, roots, litter and humus, and A-horizon soil horizon), taxa richness for plants (species) and A-horizon oribatid mites (species) and plant-feeding and plant-associated nematodes (taxa) as proportion of maximum (c, d) and soil nutrient pools (e, f) under increasing *Kunzea* (a, c, e) and *Pinus* (b, d, f). Inset figure in a and b is C loss from grasses, rescaled to show significant, but quantitatively-small, loss. All regression lines are significant at $P < 0{\cdot}05$, except for the loss of non-tree biomass C at Avoca (a) which is $P = 0{\cdot}051$. Results are selected to highlight consistent trends across the two sites; more complete results including regression coefficients given in Tables 1 and S2 Supporting Information.

Table 1. Selected carbon, biodiversity and nutrient responses to *Pinus nigra* and *Kunzea ericoides* successions, giving site means, the best model (lowest AIC) and model coefficients, highlighting consistent results across both successions. An expanded version of this table is given in Table S2 in Supporting Information

Site	Mean	Units	Best model	Model (x = tree biomass, kg m^{-2})	P	R^2
Total carbon (tree biomass + roots + soil + non-tree biomass)						
Pinus	8·8	kg C m^{-2}	Linear increase	$5{\cdot}1 + 0{\cdot}56x$	<0·001	0·93
Kunzeas	6·4	kg C m^{-2}	Linear increase	$4{\cdot}5 + 0{\cdot}52x$	<0·001	0·80
Plant-species richness						
Pinus	39	Species	Quadratic	$37 + 1{\cdot}8x - 0{\cdot}1x^2$	0·0092	0·47
Kunzea	45	Species	Quadratic	$26 + 8{\cdot}8x - 0{\cdot}57x^2$	<0·001	0·93
Plant-feeding and plant-associated nematode richness						
Pinus	3·8	Taxa	Log decrease	$5{\cdot}9 - 1{\cdot}4 \log(x + 1)$	<0·001	0·68
Kunzea	2·9	Taxa	Log decrease	$4{\cdot}3 - 1{\cdot}1 \log(x + 1)$	0·0035	0·45
Oribatid mite richness (soil A-horizon)						
Pinus	7·0	Taxa	Log decrease	$10 - 2{\cdot}1 \log(x + 1)$	0·0035	0·45
Kunzea	6·3	Taxa	Log decrease	$9 - 2{\cdot}1 \log(x + 1)$	0·041	0·23
Soil C : N ratio						
Pinus	17		Linear increase	$16 + 0{\cdot}15x$	<0·001	0·75
Kunzea	16		Linear increase	$14 + 0{\cdot}29x$	0·0050	0·43
Second NaOH organic-P						
Pinus	73	mg kg^{-1}	Log decrease	$99 - 17 \log(x + 1)$	<0·001	0·71
Kunzea	41	mg kg^{-1}	Linear decrease	$46 - 1{\cdot}6x$	0·0078	0·39
Residual-P						
Pinus	180	mg kg^{-1}	Linear decrease	$200 - 2{\cdot}7x$	0·041	0·23
Kunzea	160	mg kg^{-1}	Log decrease	$200 - 29 \log(x + 1)$	0·023	0·29

bacterial-feeding and omnivorous nematodes in the A-horizon also declined with *Pinus* biomass (Table S2). Similar to plant-feeding nematodes, oribatid mite taxa richness declined rapidly with increasing *Pinus* or *Kunzea* (Fig. 2; Table 1). Total mite taxa richness in the A-horizon also declined with *Pinus*, but not *Kunzea* (Table S2), although there was no significant loss of mite richness with *Pinus* biomass when litter and A-horizon were considered jointly (not shown).

Microbial functional diversity, as measured by CRP diversity (H′), declined with increasing *Pinus* but not with *Kunzea* biomass. As tree biomass increased, both sites showed significant changes in microbial community functioning, as shown by increases in the proportional respiration response to carboxylic acids, and decreases in the proportional response to amino acids (not shown). There was no significant change in PLFA diversity (H′) or either bacterial or fungal T-RFLP peak numbers at either site.

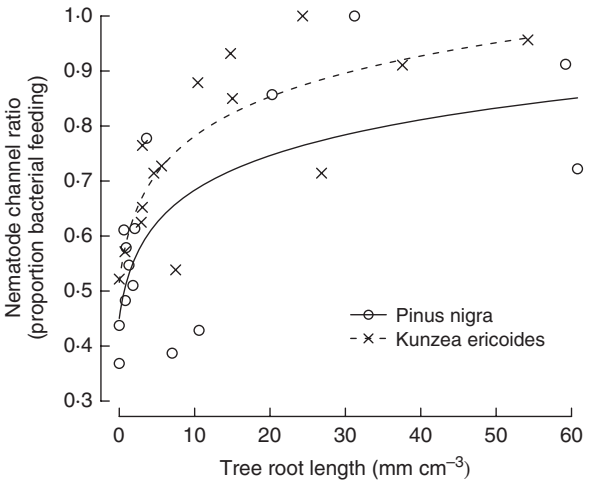

Fig. 3. Increasing nematode channel ratio [bacterial feeders/(bacterial feeders + fungal feeders)] with *Pinus* (circles) and *Kunzea* (crosses) root length.

SOIL NUTRIENTS

Both sites showed similar, significant, increases in soil C:N ratios with increasing tree biomass, with Mt. Barker also having a significant decline in N:P ratios. Total nitrogen, NH_4-N and NO_3-N declined with *Pinus* biomass at Mt. Barker, but showed no change at Avoca (Table S2). The relative size of different P pools in sequential fractionation was influenced by tree biomass at both sites (MANOVA Mt. Barker $P = 0{\cdot}0042$ and Avoca $P = 0{\cdot}025$). This was driven by declines in the two most recalcitrant-P pools (second NaOH-extracted organic-P and residual-P) at both sites (Fig. 2e,f; Table 1) and significant declines in first NaOH-extracted organic-P and increases in two labile inorganic-P fractions ($NaHCO_3$-extracted inorganic-P and first NaOH extracted inorganic-P) with *Pinus* at Mt. Barker (Table S2). Total-P and pH also decreased with *Pinus* biomass at Mt. Barker, but not with *Kunzea* at Avoca (Table S2).

FUNGAL VS. BACTERIAL DOMINANCE

Phospholipid fatty acid and hyphal length measurements showed few changes with increasing tree density. Counter to our expectation, not only was there no evidence of increased fungal biomass, but there was a marginally significant ($P = 0{\cdot}07$) *decline* in the proportion of fungal PLFAs relative to bacterial PLFAs with *Pinus* biomass at Mt. Barker. Direct measures of fungal hyphal length showed no significant effect of tree dominance on total hyphal length or arbuscular mycorrhizal hyphal length at either site. The PLFA marker 16:1ω5, a putative indicator of arbuscular mycorrhizal fungi, declined with *Pinus* biomass ($P = 0{\cdot}0013$) but not with *Kunzea* biomass.

Despite no change in fungal or bacterial biomass, soil energy channel (food web) analysis showed a shift away from fungal energy channels and towards increasingly bacterial energy channels with tree succession. There was a significant increase in nematode bacterial energy pathways relative to fungal pathways with increasing *Pinus* at Mt. Barker, with a similar marginally significant trend under *Kunzea* at Avoca ($P = 0{\cdot}059$). When tested against tree root length rather than tree biomass, both sites showed a significant increase in bacterial energy pathways relative to fungal pathways with increasing tree-root length in soils (Fig. 3). Oribatid mites, which are predominately fungal feeders, also showed significant declines in abundance at both sites with increasing tree biomass (Table S2). In contrast, Collembola, which predominately eat fungi, increased with *Pinus* biomass at Mt. Barker but showed no change at Avoca.

Discussion

Substantial increases in C sequestration associated with both *Pinus* and *Kunzea* succession in grasslands were associated with major shifts in soil nutrient pools and microbial functioning and complex changes in the richness of different taxonomic groups. While some taxonomic groups showed little change in richness with tree succession (e.g. fungal and microbial diversity), the richness of other taxa showed either strong declines even at low tree densities (e.g. oribatid mites, plant feeding nematodes), or at least initial increases (e.g. plants). These results highlight the challenge of managing for different aspects of ecosystem services during land cover change.

More pronounced effects were observed under *Pinus* succession than under *Kunzea*, but there were a number of similarities including the shift to increasingly bacterial relative to fungal energy channels, increased soil C:N ratio and declines in recalcitrant organic-P pools. Congruent patterns may indicate general tree succession effects, not dependent on native vs. exotic status, nor specific to just one site. Differences between sites often reflected significant *Pinus* effects, with no corresponding effect of *Kunzea*. More pronounced effects of *Pinus,* despite *Pinus* having similar canopy cover and being less than half the age of *Kunzea*, were probably driven by its greater growth rate and biomass and, potentially, litter quality differences, but our

design does not permit species-effects to be separated from other site differences.

CARBON AND SOIL COMMUNITIES

Woody succession caused large increases in total ecosystem C pools regardless of tree species, with the quantity of C fixed by the non-native *Pinus* greatly exceeding the native *Kunzea* (see Trotter *et al*. 2005). Virtually all of the increases in C pools were in above-ground biomass, and more than compensated for losses from other pools during woody succession. Nonetheless, there were intriguing effects of both tree species on cycling of C and organic nutrients in the soil.

Trees influence soil-C cycling by changing microclimate including soil temperature, removing water and nutrients, altering quantity and quality of litter and root C inputs, and by altering soil biota (Binkley & Giardina 1998). A 40% increase in soil respiration rates under low densities of *Pinus* trees probably reflects increased C inputs, as supported by the correlation of soil respiration with *Pinus* leaf area. The lack of any significant effect on hyphal lengths or fungal and bacterial PLFA markers suggests that increased C input from trees did not result in an increase in total fungal or bacterial biomass. Rather, we observed a significant increase in bacterial energy channels relative to fungal channels. This is counter to our expectation (see Lauber *et al*. 2008; Harris 2009) and reports from pasture–pine-plantation comparisons (Chen, Condron & Xu 2008; Macdonald *et al*. 2009). The increase in bacterial energy channels was not reflected in an increase in bacterial biomass as measured by PLFA. Unlike fungi, bacteria are primarily under top-down control by grazers (Bardgett & Wardle 2010). Increased C inputs to the bacterial channel may therefore be primarily expressed at higher trophic levels, reflecting an increased turnover rate of the bacterial pool, rather than an increase in the size of that pool. A high ($>0{\cdot}8$) NCR was also reported by Dehlin *et al*. (2008) for relatively young *Pinus* and *Pseudotsuga* plantations compared with adjacent native *Nothofagus* forest. The increased bacterial energy channel may be driven by ectomycorrhizal priming of bacterial communities (Finlay 2008), as suggested by a stronger correlation of bacterial energy channels with tree root length than tree biomass.

BIODIVERSITY

Removal of woody plants, particularly exotic invaders, in grasslands has been justified on the basis of biodiversity protection (Richardson & Rejmánek 2004). Our results provide mixed support, with rapid declines in taxonomic richness of some groups of soil invertebrates, but other groups such as plants showed at least initial increases in species richness while some groups (e.g. fungi) showed little change.

One factor that may have contributed to the rapid loss of soil invertebrate taxonomic richness was the rapid loss of grass biomass. Grasses have relatively high quality above-ground litter and abundant fine roots that provide a food resource and a habitat for soil invertebrates. The decline of particular functional groups during woody succession, such as grasses, may be more important than plant diversity in explaining soil faunal diversity (De Deyn *et al*. 2004; Viketoft *et al*. 2009). The creation of a novel habitat in *Pinus* litter may partially compensate for the loss of mite richness in the A-horizon, as there was no significant loss of mite richness with *Pinus* biomass when litter and A-horizon were considered jointly (with the possible caveat that different extraction methods were used). Nonetheless, the mite fauna colonizing *Pinus* litter was largely a novel community (54% of mite taxa in *Pinus* litter were not found in the A-horizon) rather than taxa moving up out of the A-horizon in the presence of *Pinus* litter (only 15% of species did so).

Initial increases in plant-species richness were reversed at higher tree densities under *Pinus*. These initial increases are likely to be ephemeral in the absence of management, as intermediate *Pinus* stand densities are likely to rapidly promote conspecific recruitment and attain high density within a further 20 years. Management to prevent canopy closure of low density *Pinus* stands may maintain diversity of some groups, including plants and above-ground invertebrates (see Pawson *et al*. 2010), but is unlikely to maintain diversity of key soil functional groups (plant-feeding and plant-associated nematodes, oribatid mites) which showed strong negative effects of even low density *Pinus*.

NUTRIENTS AND SOIL COMMUNITIES

We expected changes in soil nutrients with tree litter inputs and the establishment of ectomycorrhizal fungi associated with trees. This expectation was supported by an increase in the C:N ratio of soil, shifts in soil P pools, and, under *Pinus*, a significant loss of soil-C (see Chapela *et al*. 2001; Guo & Gifford 2002). Ectomycorrhizal fungi have been implicated in increased mineralization of soil organic-P fractions (Scott & Condron 2003; Chen, Condron & Xu 2008) and release of soil-C (Chapela *et al*. 2001). The increase in soil C:N ratios and decline in recalcitrant organic-P pools are consistent with the effects of organic nutrient uptake by ectomycorrhizal fungi associated with both *Pinus* and *Kunzea* (Read, Leake & Perez-Moreno 2004). Organic nutrient uptake by ectomycorrhizal fungi may, in turn, have driven or contributed to the significant shifts in microbial catabolic responses, as utilization of amino acids by ectomycorrhizal fungi may reduce amino acid utilization by free living microbes (Degens & Harris 1997). Nonetheless, both above-ground (litter, shading) and below-ground (roots, ectomycorrhizas) effects are likely to contribute to the observed changes in soil nutrient cycling and soil communities.

APPLICATIONS

Several studies have compared *Pinus* plantations to adjacent grasslands as a surrogate for invasion (e.g. Chapela *et al*. 2001; Pawson *et al*. 2010). Our results suggest that studying self-established invasive trees may enhance understanding of this spatially heterogenous process. In particular, nonlinear responses to tree density might have been missed in uniformly planted trees. Including comparisons of non-native to native

woody-successions also provides a valuable context. While our study was not designed for statistical comparisons of *Pinus* vs. *Kunzea*, it was clear that there is much lower C sequestration but higher plant biodiversity associated with *Kunzea* than *Pinus*. Despite much stronger effects of *Pinus* than *Kunzea*, there were also parallels in the response of soil communities and ecosystem processes.

While it has been suggested that low tree-densities have only minor effects on biodiversity based on responses of beetles (Pawson *et al*. 2010), our results suggest that the density effect of trees on biodiversity depends on which taxa are measured. For example, only closed-canopy *Pinus* resulted in lower plant-species richness than the initial grasslands and we observed no negative effect of *Kunzea* on plant-species richness. Nonetheless, succession from grasslands to *Pinus* or *Kunzea* caused rapid declines in taxonomic richness of key soil invertebrate groups. Therefore, our finding of divergent responses of plant and soil invertebrate species richness runs counter to the indicator species concept, suggesting land managers should consider multiple taxonomic groups in assessing biodiversity impacts. Further, strong recommendations have been made that restoration ecologists should target increased fungal dominance early in restoration (Harris 2009). Our finding of increased bacterial energy channels in woody succession suggest that making recommendations about desirable soil communities during restoration may be premature.

Carpenter *et al*. (2009) noted the importance of making trade-offs between ecosystem services explicit. Achieving closed-canopy *Pinus* forest may appear to be a desirable outcome for New Zealand ecosystems, from a purely C-based view. This comes, however, at significant costs in terms of biodiversity and complex effects on soil nutrient cycling.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Soil respiration responses to biomass and tree leaf area under *Pinus* succession.

Table S1. Additional site-level information including soils and vegetation.

Table S2. C, biodiversity, and nutrient responses to *Pinus* and *Kunzea* successions.

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