100 YEARS Journal of Ecology British Ecological Society

Journal of Ecology 2014, **102**, 622–631 doi: 10.1111/1365-2745.12234

Soil-mediated effects of invasive ungulates on native tree seedlings

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Summary

1. Invasive browsing ungulates can have strong impacts on the structure and composition of forest ecosystems, particularly where ungulates are not native ecosystem components as in New Zealand. Ungulate impacts on plant communities have been considered mostly from an above-ground perspective. However, understanding below-ground effects of these invasive herbivores is critical as they may drive feedbacks to above-ground ecosystem components.

2. We measured growth responses of seedlings of five common tree species in a greenhouse experiment in soils collected from 26 plots fenced to exclude invasive ungulates for at least 17 years and from paired, unfenced control plots. We then further investigated soil-mediated effects of ungulates on one tree species, *Melicytus ramiflorus*, by partitioning these effects into soil abiotic and biotic components, as well as measuring arbuscular mycorrhizal fungal (AMF) root infection.

3. Biomass of seedlings of all five species was greater in soils from within exclosures, although this was only significant for two species. These soil-mediated effects were partially driven by changes in physical and chemical soil properties; soil bulk densities were lower inside exclosures than in controls.

4. Effects of invasive ungulates on seedling biomass of *M. ramiflorus* were positively related to effects on per cent AMF root infection. The biomass of *M. ramiflorus* seedlings was positively related to the AMF infection of its roots, which in turn was related to greater organic matter content and lower bulk density of soils from within exclosures. Results for *M. ramiflorus* indicated that soil-mediated effects of ungulates on seedling biomass were of abiotic origin, but were mediated by the biotic soil component, that is, through effects on AMF.

5. *Synthesis.* Invasive herbivores may potentially impact on plant performance and community structure not only directly but also indirectly through influencing soil abiotic and biotic properties. Our results show that shifts in plant–soil interactions and feedbacks represent important but understudied pathways by which invasive ungulates can have wide-ranging impacts on forest ecosystems. Future studies should consider the importance of soil-mediated effects of invasive ungulates relative to direct effects of herbivory.

Key-words: above-ground–below-ground interactions, arbuscular mycorrhizal fungi, biological invasions, browsing mammals, cluster roots, deer, feral goats, herbivory, New Zealand forests, soil organisms

Introduction

Ungulates frequently become invasive and attain large population sizes when introduced to regions from which they have been previously absent (Caughley 1970). These introductions have been shown to exert strong impacts on the functioning of ecosystems (Vazquez 2002; Wardle & Bardgett 2004). Effects of browsing ungulates on ecosystem properties are often mediated through shifts in the productivity and composition of plant communities (Pastor *et al.* 1993; Augustine & McNaughton 1998). Hence, unravelling the mechanisms through which invasive ungulates impact on plant communities is important for understanding the basis of ungulate

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ecosystem impacts. Most prior studies on the ecological impacts of ungulates (whether native or invasive) have focussed on the direct effects of these browsers on plant communities, through removing above-ground plant biomass and changing plant species composition (e.g. Augustine & McNaughton 1998; Wardle *et al.* 2001; Côté *et al.* 2004). Ungulates can also affect plant performance indirectly through shifts in soil properties; however, these pathways have not been well studied.

Ungulates can directly alter soil properties through physical soil disturbance, such as compaction resulting from trampling (Duncan & Holdaway 1989). For example, intensive grazing by red deer (*Cervus elaphus scoticus*) increased soil bulk density by 25% over 15 years (Gass & Binkley 2011). Further, ungulates can consume large amounts of plant material and may thereby promote soil microbial activity and enhance decomposition and nutrient mineralization rates when resources are returned to the soil in a labile form as faeces (Bardgett, Wardle & Yeates 1998; Van der Wal *et al.* 2004). Ungulates can also affect soil properties via shifts in plant nutrient and carbon (C) allocation strategies (Hamilton & Frank 2001; Ayres *et al.* 2004), in turn increasing microbial activity (Hamilton *et al.* 2008). Over longer time frames, ungulates can further alter soil properties through causing shifts in plant species composition (St. John *et al.* 2012). Selective feeding on nutrient-rich plants often shifts competitive interactions among plants by favouring species of low palatability (e.g. Wardle *et al.* 2001; Husheer, Allen & Robertson 2006; Barrios-Garcia, Relva & Kitzberger 2012). The same traits that confer low palatability to plants (i.e. high concentrations of structural carbohydrates and chemical defence compounds, low nutrient concentrations) also result in poor-quality litter that slowly decomposes, thus reducing nutrient mineralization rates (Grime *et al.* 1996; Wardle, Bonner & Barker 2002; Harrison & Bardgett 2004).

Taken together, effects of ungulates on abiotic and biotic soil properties have the potential to impact either negatively (e.g. Sørensen *et al.* 2008) or positively (e.g. Hamilton & Frank 2001; Hamilton *et al.* 2008) on plant performance, with possible consequences for the long-term functioning of the ecosystem. Such soil-mediated effects of large herbivores on plant performance have been suggested for (overabundant) ungulates in their native range (e.g. Habeck 1960; Augustine, McNaughton & Frank 2003), but empirical tests are lacking. Moreover, whether and how the effects of invasive ungulates on soil properties feed back above-ground in ecosystems historically free from mammalian herbivores (e.g. many island ecosystems) remains entirely unexplored.

Here, using a controlled experiment allowing us to isolate indirect effects from direct effects, we explicitly tested for soil-mediated effects of invasive ungulates on plant performance in New Zealand temperate rain forests. New Zealand forests were colonized by ungulates when several large species, notably European red deer and goats (*Capra hircus*), were introduced between the late 18th and early 20th century; there are no native ungulates or other mammalian browsers in New Zealand. Previous work has shown that widespread shifts in the understorey vegetation composition of New Zealand forests caused by invasive ungulates have led to an alteration of several soil abiotic and biotic properties (Wardle *et al.* 2001). For the present study, we collected soils from 26 long-term (17–50 years) fenced ungulate exclosure plots (and paired, unfenced control plots) scattered throughout the temperate rain forest of northern New Zealand (Fig. 1 and Table S1) to explore soil-mediated mechanisms by which invasive ungulates could affect plant performance.

First, we tested the hypothesis that invasive ungulates affect soil properties which in turn affect relative plant performance. In a greenhouse experiment, we measured growth responses for seedlings of five tree species, all of which are commonly

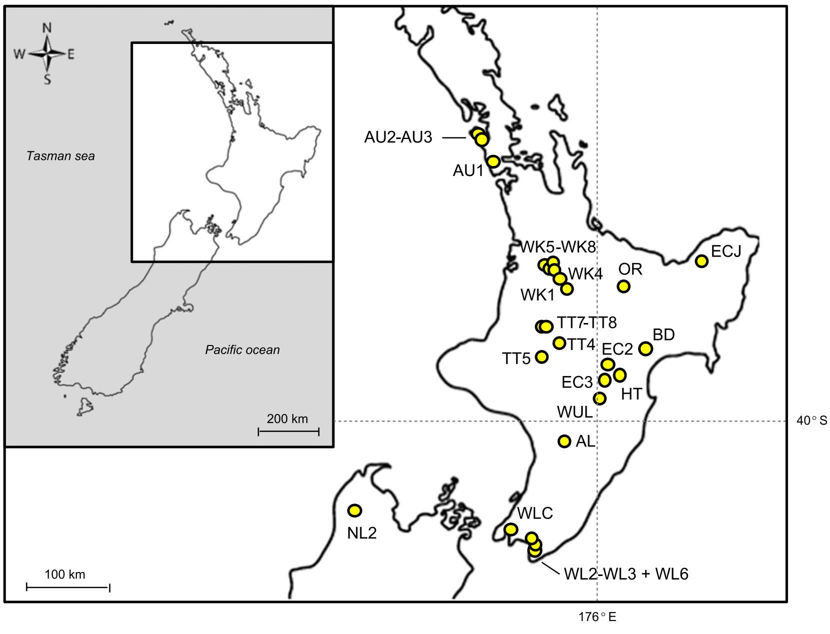

Fig. 1. Location of the 26 long-term exclosure plots (and paired control plots) across temperate rain forests in the northern part of New Zealand. Inset shows New Zealand's North and South Islands. Details of each site are in Table S1.

found in New Zealand temperate rain forests but have contrasting functional traits (Table S2). The effects of ungulates on soil properties in these forests are highly variable (Wardle *et al.* 2001), so we tested whether the soil-mediated effects of ungulates on plant growth could be explained by site characteristics and/or the effects of ungulates on soil abiotic properties. Secondly, for a subset of the sites, and focusing on *Melicytus ramiflorus*, which is one of the most widespread and abundant palatable tree species in these forests, we partitioned soil-mediated effects of ungulates on plant growth into soil abiotic and soil biotic components using soil sterilization and re-inoculation. We tested the hypothesis that both soil components, either additively or interactively, contribute to the net soil-mediated effects of ungulates on plant performance. We measured both plant biomass response and arbuscular mycorrhizal infection, to test indirect effects via modification of soil symbionts. Exploring these novel pathways through which ungulates can affect plant performance advances our understanding of the mechanistic basis of the community and ecosystem impacts of invasive animals.

Materials and methods

SITES AND SAMPLING DESIGN

We selected 26 ungulate exclosures based on the following criteria: (i) located in a region known to have introduced ungulates, (ii) having a fence established and secure for at least 15 years and (iii) having a nearby, paired unfenced control plot. These plots represent a wide geographic range and are scattered throughout different New Zealand forest types (Fig. 1 and Table S1). Plots were sampled in *Nothofagus*–broad-leaved–podocarp forests, broad-leaved–podocarp forests and shrublands; further, one plot was in pure *Nothofagus* forest (Wiser *et al.* 2011; Wiser & De Cáceras 2013; vegetation alliances sampled are listed in Table S1). Across sites, dominant tree species included gymnosperms of the Podocarpaceae, broad-leaved angiosperms and tree ferns (Cyatheaceae and Dicksoniaceae). Introduced ungulates in the New Zealand temperate rain forests where our exclosure plots are located include European red deer (*Cervus elaphus scoticus*), fallow deer (*Dama dama*), sika deer (*Cervus nippon*) and feral goats (*Capra hircus*). Additional introduced mammals include a range of rodents and mustelids as well as the brushtail possum (*Trichosurus vulpecula*); these animals were not excluded by the fences. These exclosure plots are approximately 20 × 20 m, surrounded by a 2-m-tall fence, and each exclosure is paired with a same size unfenced control plot; each exclosure and its control are hereafter referred to as a ‘site’. Most exclosures were established by the former New Zealand Forest Service in the 1980s (Table S1). Between November 2009 and January 2010, all plots were assessed for vegetation composition using the permanent plot measurement techniques described by Hurst and Allen (2007). Diametre at breast height (DBH) for all stems ≥2.5 cm was measured, and all tree saplings were counted. At the same time, in each plot, 16 soil cores (6.5 cm diametre, 10 cm depth) were collected: four cores from each quadrant, at 90° from each other, and one metre from the centre of the quadrant (Stevenson & St. John 2009). For each plot, soil cores were bulked and transported to the laboratory where they were stored at 4 °C before processing. Subsamples were analysed for physical and chemical soil properties using standard methods: bulk density, pH, soil organic matter, total soil C using a Leco FP-2000 Analyzer, total N (Kjeldahl method), P (as described by Jackson (1958)) and soil available P (Bray-II; Bray & Kurtz 1945).

GREENHOUSE EXPERIMENTS

Experiment 1 tested the hypothesis that invasive ungulates affect soil properties which in turn influence plant relative performance, using soil from each of the 26 sites to test for overall soil-mediated effects across multiple plant species. For each plot, soils were homogenized, sieved through 1-cm mesh to remove large stones and coarse roots (>4 mm diameter) and mixed with sterilized (autoclaved) sand in a 1:1 volumetric ratio. Roots < 4 mm diameter retained on the 1-cm mesh were cut into 1 cm lengths and re-added to the soils; this permitted more uniform distribution of root fragments across treatments. For each plot, five 500-mL pots were filled with an equal volume of the soil–sand mixture and planted with one seedling of one of the following angiosperm tree species: *Coprosma robusta* (Rubiaceae), *Fuchsia excorticata* (Onagraceae), *Knightia excelsa* (Proteaceae), *Melicytus ramiflorus* (Violaceae) or *Pittosporum crassifolium* (Pittosporaceae). These five species were selected because of their contrasting trait characteristics (Table S2). *Weinmania racemosa* was also initially included, but had very low survival and growth and was therefore excluded from further analysis. Commercially provided seeds (New Zealand Tree Seeds, www.nzseeds.co.nz) were germinated in sterilized vermiculite in a room-temperature laboratory. Experiment 1 consisted of two soil treatments (i.e. exclosure or control) × 26 sites × 5 plant species = 260 experimental units.

The second hypothesis that both abiotic and biotic soil components contribute to soil-mediated effects of invasive ungulates on plant performance was tested in Experiment 2. This experiment was run for a single plant species (*M. ramiflorus*), using soils from 12 of the 26 selected sites (Table S1). We chose *M. ramiflorus* because it occurred at 19 of the 26 sites (73% of sites) and previously has been shown to be negatively affected by introduced browsing mammals (Wardle *et al.* 2001). This, combined with it being an arbuscular mycorrhizal species, makes it an ideal species for exploring the mechanistic basis of how browsing mammals may indirectly impact on plant performance via the soil. We differentiated between abiotic and biotic soil-mediated effects of ungulates on plant growth, using sterilized soils and living soil inocula (e.g. Kardol *et al.* 2007; Wardle *et al.* 2012). We used a paired 2 × 2 factorial design, in which for each site, we grew seedlings in sterilized soils from either the exclosure or the paired control plot, and inoculated each sterilized soil with living soil inoculum from either the exclosure plot or the paired control plot. Soils were sterilized by γ-irradiation (25 kGy; Schering Plough Animal Health Limited, Wellington, New Zealand), which is widely used for killing soil biota in plant–soil feedback experiments (e.g. Kardol *et al.* 2007). The bottom of the 500-mL pots was filled with 125 mL sterilized sand. The pots were then filled with a mixture consisting of 50.0% sterilized sand, 43.3% sterilized soil and 6.7% living soil inoculum, such that the total volume of living soil inoculum per pot was 5%. The soil inoculum potentially includes all soil biota (prokaryotes, fungi, animals). All pots were planted with one seedling of *M. ramiflorus*. Experiment 2 consisted of two soil treatments (i.e. exclosure or control) × 2 inoculation treatments (i.e. live exclosure or control soil) × 12 sites = 48 experimental units.

We performed our study under controlled conditions, because we were explicitly interested in isolating the indirect, soil-mediated effects of the invasive ungulates, which would be exceedingly challenging in the field. This is consistent with what has been done in other studies that have shown how soil-mediated effects of aboveground consumers influence plant performance (Sørensen *et al.* 2008; Wardle *et al.* 2012). Plants were grown in a greenhouse at Landcare Research in Lincoln, New Zealand (43°38′S, 172°29′E), from mid-March to early November 2010. The greenhouse was heated during the winter months to avoid frosting tender seedlings; otherwise, the greenhouse air temperature was within 5 °C of the ambient temperature. Plants were watered one to three times per week. All pots were isolated in individual saucers on a free-draining, open-mesh bench to prevent any cross-contamination via water movement. On 4 September 2010, a M_W 7.1 earthquake toppled most pots, but damage to seedlings was limited, and while minor soil mixing occurred, it was near the final harvest and hence unlikely to have altered final growth results. At harvest, plant shoots were clipped at the soil surface, and roots washed from the soil. Shoot and root dry mass were determined after drying at 60 °C for at least 72 h.

MYCORRHIZAL ROOT INFECTION AND CLUSTER ROOTS

For roots of each *Melicytus ramiflorus* seedling (both in Experiment 1 and Experiment 2), we estimated the percentage of root length infected by arbuscular mycorrhizal fungi (AMF) using a line intersection method (McGonigle *et al.* 1990). For each sample, after roots were washed from the soil (see above), a 0.5 g fresh weight subsample of randomly selected fine root segments was placed in histocassettes and stored in 70% ethanol for at least 24 h. Roots were placed in 10% KOH and cleared by double autoclaving (2 × 30 min cycles), rinsed with water, acidified in 5% HCl for 5 min, then placed in trypan blue stain (40% glycerol, 20% lactic acid, 0.01% trypan blue) overnight. Roots were then de-stained (40% glycerol, 20% lactic acid) for at least 24 h and mounted on slides. Using a compound microscope, slides were scanned and when vertical cross-bars intersected a root, mycorrhizal presence or absence was recorded until 100 hits were recorded.

For roots of each *K. excelsa* seedling in Experiment 1, we estimated the relative abundance of ‘cluster roots’ (also called ‘proteoid roots’) by using a line intersection method. Cluster roots are thought to improve nutrient acquisition, notably phosphate absorption (Lambers *et al.* 2003; Shane & Lambers 2005). After washing from soil (see above), roots were placed in a Petri dish and overlaid with a transparent grid-lined cover (2.5 × 2.5 mm). Under magnification, we visually assessed all intersections of roots with grid lines. For each hit, we scored the presence or absence of cluster roots. We distinguished between living (‘light’) and dead (‘dark’) cluster roots.

DATA ANALYSES

Treatment effect on seedling biomass (total, root, shoot) in Experiment 1 was analysed by linear mixed effect models using the lmer function from the lme4 package in R (version 2.12.1), which included plant species (*C. robusta*, *F. excorticata*, *K. excelsa*, *M. ramiflorus*, *P. crassifolium*), soil treatment (exclosure vs. control) and their interaction as fixed factors and site as a random factor. We used a model simplification approach (Crawley 2007), starting from a maximum model including all explanatory factors. Factors to be tested were then removed one at a time; we then compared the depleted model with the previous one using an ANOVA function. Effects of tested factors were considered significant if the *P*-value for the ANOVA test was < 0.05.

For biomass responses for each plant species separately, soil treatment effects were tested in a similar way by comparing models with or without including soil treatment (exclosure vs. control) as a fixed factor, and site as a random factor; responses of AMF root infection for *M. ramiflorus,* and of total root length and total and percentage length of cluster roots for *K. excelsa* seedlings, were tested similarly. Effects of ungulates on soil properties measured on field-collected soils were analysed similarly by comparing models with or without including treatment (exclosure vs. control) as a fixed factor.

To facilitate visual interpretation of across-species comparisons of exclosure effects (Fig. 2), biomass data for each species were standardized by subtracting the mean species biomass from an individual datum and then dividing by the standard deviation (Mulder *et al.* 2004). Then, across species, biomass data were normalized by subtracting the site mean biomass to make it possible to compare exclosure effects across sites with different intrinsic soil fertility.

In order to explain observed across-site variation in soil-mediated effects of ungulates on total seedling biomass in Experiment 1, stepwise multiple regressions were run using the magnitude of effects as dependent variables, and with each site representing an independent data point. Magnitudes of effects were calculated as (contr-excl/max (contr,excl)) following Diez *et al.* (2010), where contr = biomass on soils from control plots and excl = biomass on soils from exclosure plots. The maximum biomass is used in the denominator to avoid a potential bias towards negative values. Regressions were run separately for each plant species. A first series of regressions was run to test relationships between the effects of ungulates on seedling biomass and the effects of ungulates on abiotic soil properties as observed in the field. Here, the initial model included the following variables: soil organic matter content, total soil C content, total soil nitrogen (N) content, total soil phosphorus (P) content, soil available P (Bray-II), pH and soil bulk density. Similar regressions were run to test the relationships between effects of ungulates on per cent mycorrhizal root infection and the effects of ungulates on abiotic soil properties. A second series of regressions was run to test relationships between the effects of ungulates on seedling biomass and site characteristics. Here, the initial model included the following variables: altitude, mean annual temperature, mean annual precipitation, soil

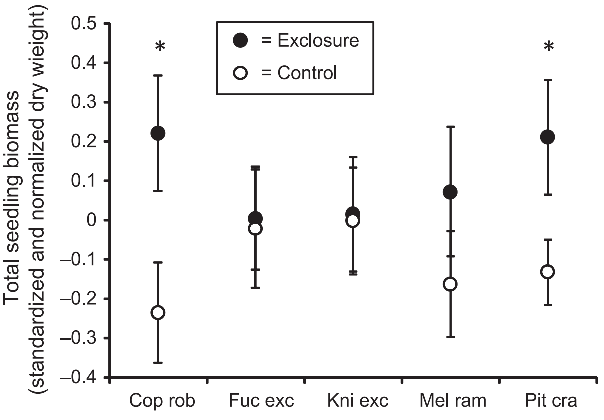

Fig. 2. Soil-mediated effects of invasive ungulates on total biomass for each of five native New Zealand tree species. Seedlings were grown in soils from inside and outside long-term exclosures. To facilitate comparisons, biomass data were standardized and normalized (see Materials and methods). Data shown are mean ± SE ($n = 26$). *Indicates significant exclosure effect ($P < 0.05$) as tested by linear mixed models (see Materials and methods). Cop rob = *Coprosma robusta*, Fuc exc = *Fuchsia excorticata*, Kni exc = *Knightia excelsa*, Mel ram = *Melicytus ramiflorus*, Pit cra = *Pittosporum crassifolium.*

particle size, total basal area in control plots (as a measure for total standing biomass) and (as a proxy for forest type) sample scores for the first two axes from principal component analysis (PCA) summarizing the tree species composition of the control plots. Together, the first two PCA axes explained 51.2% of the variation in species composition. This PCA was based on basal area for each recorded tree species per plot. For both series of regressions, the most parsimonious models were found by selecting variables by a backward selection procedure based on Akaike information criteria (AIC) using the stepAIC function from the MASS package in R. The relationship between effects of ungulates on seedling biomass and on percentage AMF root infection for *M. ramiflorus* seedlings in Experiment 1 was tested using Pearson's correlation coefficients using each site as an independent data point.

Treatment effects on biomass (total, root, shoot) and AMF root infection of *M. ramiflorus* seedlings in Experiment 2 were analysed by linear mixed models similar to the ones described above, and which included sterilized soil treatment (exclosure vs. control), living soil inoculum treatment (exclosure vs. control) and their interaction as fixed factors, and site as a random factor.

Table 1. Summary of exclosure effects on seedling biomass in Experiment 1. Effects were tested using linear mixed effect models (lmer function in R), contrasting the full model (biomass as a function of treatment, including a random term for Site; 4 d.f.) versus the null model (random site effect only; 3 d.f.) with AIC (Akaike's information criterion) values for both models

	AIC			
Species	Treatment	Null model	χ^2	*P*-value
Coprosma robusta	114.49	117.09	4.60	**0.032**
Fuchsia excorticata	135.35	133.48	0.13	0.721
Knightia excelsa	97.60	95.61	0.02	0.895
Melicytus ramiflorus	76.51	75.47	0.95	0.328
Pittosporum crassifolium	63.67	65.58	3.92	**0.048**

Significant *P*-values (in bold <0.05) indicate a significant difference in plant growth between soils from exclosure plots and soils from control plots. See Table S6 for a similar test of treatment by species interaction.

Results

EXPERIMENT 1

All five species had higher total plant biomass in soils from inside exclosures than from outside exclosures, but this was statistically significant at $P = 0.05$ for only two species, that is, *C. robusta* and *P. crassifolium* (Fig. 2 and Table 1). Testing effects across species revealed that there was no significant interactive effect of the exclosure treatment and plant species on plant biomass (Table S3). Further, with minor exceptions, biomass responses for root and shoot biomass generally mirrored the responses for total biomass, and no differences were found in root-to-shoot ratios between soils from inside versus outside the exclosures (Tables S4, S5). Seedling mortality was generally low, ranging from 0% (*K. excelsa*) to 17% (*M. ramiflorus*), and was not related to the exclosure treatment (data not shown).

Soil bulk density was lower inside exclosure plots than in control plots (0.43 ± 0.05 vs. 0.48 ± 0.05 g cm^{-3}; $\chi^2 = 6.67$, $P = 0.010$). Other physical and chemical soil properties did not differ significantly between control plots and exclosure plots (Table S6). The effects of ungulates on physical and chemical soil properties generally explained statistically significant but small proportions of among-site variation in seedling biomass responses in the greenhouse experiment (multiple regressions, Table 2). For *P. crassifolium*, the effects of ungulates on seedling biomass were strongly negatively correlated only with their effects on soil bulk density. For *M. ramiflorus*, seedling biomass was also negatively related to the effects of ungulates on soil bulk density, although the effect was only marginally significant. Relations between effects of ungulates on other soil properties and effects on seedling biomass varied among plant species (Table 2). The effects of ungulates on seedling biomass were weakly related to site characteristics, with the most parsimonious models often only being marginally significant (Table

Table 2. Results from multiple stepwise regressions testing relationships between the effects of ungulates on abiotic soil properties as observed in the field and the effects of ungulates on total seedling biomass as observed in the greenhouse experiment (Experiment 1). Magnitudes of effects were calculated as (control-exclosure)/max(control,exclosure) (see Materials and methods). The most parsimonious model was found by selecting variables using a backward selection procedure based on Akaike information criteria (AIC) using the stepAIC function in R. Values of *t* are shown for each selected variable, along with adjusted r^2 for the regression models, and *P*-values associated with the regressions. For each species, the presented models only contained those variables for which *t*-values are shown

	Variables in relationship; effects of ungulates on:											
Plant species	SOM (%)	Total C (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	P-Bray (mg kg^{-1})	pH	Bulk density (g cm^{-3})	d.f.	AIC	r^2	*F*	*P*-value
Coprosma robusta		2.6*	−1.9^{+}		−1.9^{+}			17	−32.39	0.39	5.18	**0.010**
Fuchsia excorticata		−1.5	1.4		1.3	−2.5*		21	−37.66	0.19	2.49	0.074
Knightia excelsa	−3.0**	2.5*		−1.6	2.2*				−56.26	0.28	3.43	**0.026**
Melicytus ramiflorus	−1.7						−1.9^{+}	18	−16.92	0.10	2.17	0.144
Pittosporum crassifolium							−3.9***	23	−45.68	0.37	15.33	**<0.001**

Significance level for selected variables: ^{+}P <0.1, *P <0.05, **P <0.01, ***P <0.001. Significant *P*-values (<0.05) associated with the regressions are in bold. SOM = soil organic matter, P-Bray = soil available phosphorus.

S7). Among climate factors, mean annual precipitation was positively related to biomass response of *F. excorticata*, while water deficit was negatively related to biomass responses of *K. excelsa*.

Total AMF root infection of *M. ramiflorus* did not differ between seedlings grown on soils from inside versus outside exclosures (model comparisons: $\chi^2 = 0.09$, $P = 0.763$). However, across sites, effects of ungulates on total seedling biomass of *M. ramiflorus* and their effects on the percentage AMF root infection of *M. ramiflorus* were strongly positively correlated (Fig. 3). Notably, in soils from all sites where ungulates had a positive effect on seedling biomass, they also had a positive effect on AMF root infection. There was a positive relationship between AMF infection and *M. ramiflorus* biomass (log–log, $r^2_{adj} = 0.08$, $P = 0.04$, d.f. = 50; data not shown). Multiple regression showed that the effects of ungulates on soil organic matter content (SOM) and bulk density explained a significant proportion of among-site variation in AMF infection (SOM: $t = -2.5$, bulk density: $t = -2.5$; d.f. = 18, AIC = -21.78, $r^2 = 0.26$, $F = 4.53$, $P = 0.025$).

For *K. excelsa*, total root length and total and percentage length of cluster roots did not differ between seedlings grown on soils from inside versus outside exclosures (model comparisons for total root length, total length of cluster roots and percentage length of cluster roots, all $P > 0.1$).

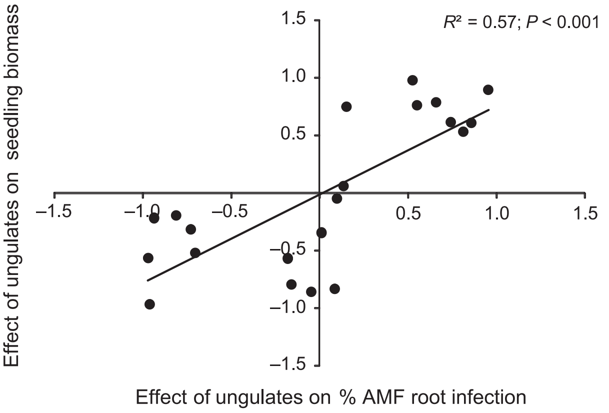

Fig. 3. Relationship between soil-mediated effects of ungulates on percentage arbuscular mycorrhizal fungal (AMF) root infection and on total biomass of *Melicytus ramiflorus* seedlings.

EXPERIMENT 2

Across living soil inocula treatments, biomass of *M. ramiflorus* was greater in re-inoculated sterilized exclosure soils than in re-inoculated sterilized control soils. (Fig. 4a and Table 3). Total biomass production was unaffected by the living soil inocula from either inside or outside exclosures, and there were no interactive effects between sterilized soil and living soil inoculum treatments (Table 3). The response for both root and shoot biomass mirrored the responses of total biomass with the exception that the effect of the sterilized soil treatment for root biomass was only marginally significant (Table S8). There was, however, a significant interactive effect for the root–shoot ratio (Table S8): the ratio was highest when the sterilized soil and the living soil inocula both came from inside exclosures or when both came from outside exclosures.

Total AMF root infection of *M. ramiflorus* was unaffected by the source of living soil inoculum. However, seedlings grown on re-inoculated sterilized soils from inside exclosures had greater AMF root infection than seedlings grown on re-inoculated sterilized soils from outside exclosures (Fig. 4b, Table 3). There was no interactive effect between the sterilized soil and living soil inoculum treatments on AMF root infection. Plant biomass was positively related to total AMF root infection (log–log transformation, $r^2_{adj} = 0.19$, $P = 0.0015$, d.f. = 46).

Discussion

By using soils from a unique network of long-term ungulate exclosures in controlled greenhouse experiments, we were able to show that the ecosystem impacts of invasive ungulates extend beyond direct effects of removing above-ground plant biomass (Bellingham & Allan 2003) to include changes in

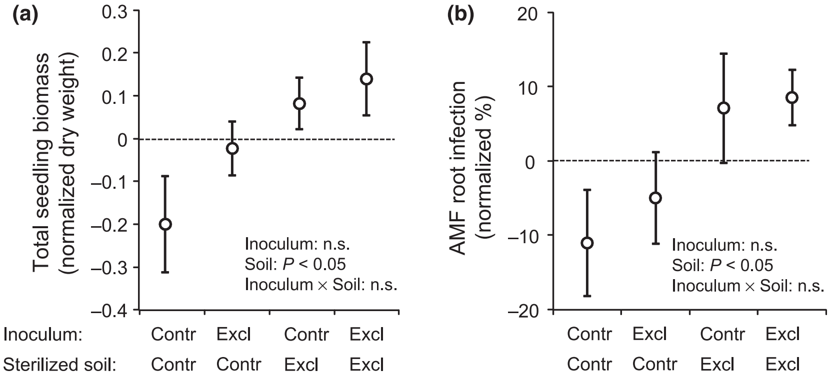

Fig. 4. Responses of total biomass (a) and arbuscular mycorrhizal fungal (AMF) root infection (b) for *Melicytus ramiflorus* seedlings grown on sterilized soils from inside exclosures or from control plots, inoculated with living soil inoculum from inside exclosures or from control plots. Data were normalized by site to make it possible to compare exclosure effects across sites that are inherently different from each other; data were brought to a common scale by subtracting the site mean from an individual standardized datum. Data shown are mean ± SE ($n = 12$). Excl = exclosure, Contr = control.

Table 3. Summary of soil treatment (S) and inoculum effects (I) on seedling biomass and AMF infection of *Melicytus ramiflorus* in Experiment 2. Effects were tested using linear mixed effect models (lmer function in R). Site was included as random factor. We used a model simplification approach, starting from the full model including all explanatory factors. Factors to be tested were then removed one at a time; we then compared the depleted model with the previous one using an anova function

Response variable	Model	Fixed factors	d.f.	AIC	Comparison	Effect tested for	χ^2	*P*-value
Biomass	1	S, I, S × I	6	74.31				
	2	S, I	5	72.69	1 vs. 2	S × I	0.38	0.563
	3	S	4	72.14	2 vs. 3	I	1.44	0.230
	4	I	4	76.06	3 vs. 4	S	5.37	**0.020**
AMF root infection (%)	1	S, I, S × I	6	464.21				
	2	S, I	5	462.36	1 vs. 2	S × I	0.15	0.701
	3	S	4	460.70	2 vs. 3	I	0.34	0.562
	4	I	4	464.97	3 vs. 4	S	4.62	**0.032**

Significant *P*-values (<0.05) are in bold.

soil properties that further reduce plant growth. Soil-mediated effects were related to shifts in abiotic rather than biotic soil properties. However, these abiotic shifts, notably increased bulk density, appear to act on seedlings by modifying soil biota such as mycorrhizal fungi (Fig. 5).

Direct effects of ungulate browsing often select for unpalatable plant species (Díaz *et al.* 2007), and thereby cause shifts in the competitive balance between palatable and unpalatable species (Beguin, Pothier & Côté 2011). Conversely, indirect soil-mediated effects on plant performance may be less predictable and not necessarily related to palatability. We included species ranging from highly unpalatable (i.e. *K. excelsa*) to highly palatable (i.e. *M. ramiflorus*, *C. robusta*) in our first experiment, but the responses were unrelated to palatability (Fig. 2). Our results indicated that seedling responses to soil-mediated effects of ungulates did not vary greatly across species, that is, we did not find significant evidence of an interaction between species and treatment (Table S3). However, the differences in effect size as illustrated in Fig. 2 suggest that indirect soil-mediated effects of invasive ungulates may alter the competitive balance between co-occurring species, and probably differently from direct effects (i.e. browsing). The contribution of shifts in soil properties to the net effect of invasive ungulates on plant performance and community composition is however still largely unknown and should be a focus of future research (see also Nuñez, Bailey & Schweitzer 2010).

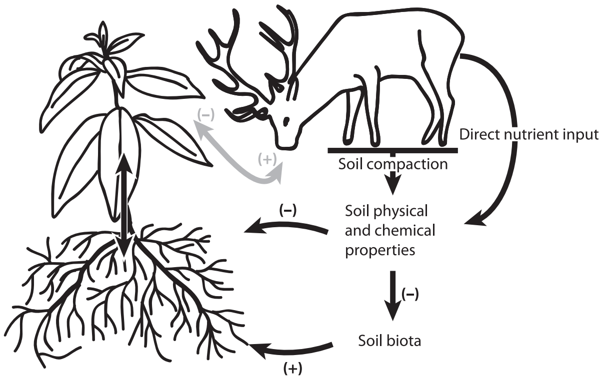

Fig. 5. Conceptual diagram of direct and indirect pathways by which (invasive) ungulates could affect plant performance. Soil-mediated responses of plant performance to ungulates may result from single or interaction effects of shifts in physical and chemical soil properties and soil biota. Ungulates can affect physical and chemical soil properties through soil compaction and through direct nutrient inputs from faeces and urine. We showed that shifts in physical and chemical soil properties negatively affected plant performance, either directly or indirectly through negative effects on mutualistic soil biota (i.e. mycorrhizal fungi). Black arrows indicate interactions addressed in this study. Grey arrows indicate interactions not addressed in this study.

Soil-mediated effects of ungulates could result from shifts in chemical and physical soil properties (e.g. Bardgett, Wardle & Yeates 1998). However, of all the soil abiotic parameters that we measured, only bulk density was significantly affected by ungulates across sites, being higher outside than inside exclosures (Table S6). Ungulates can severely alter soil structure by trampling, resulting in increased soil compaction (Habeck 1960; Duncan & Holdaway 1989; but see Frank & Groffman 1998) which could potentially restrict root penetration and thereby plant biomass production (Heckel *et al.* 2010). In that light, soil compaction could partly explain the responses of *P. crassifolium*, given that we found a strong negative correlation between ungulate effects on bulk density and their effects on *P. crassifolium* biomass. We acknowledge that processing field-collected soils for our greenhouse experiment disturbed the soil profiles, suggesting that if bulk density effects were responsible for greenhouse results, the effect may have depended on fine-scale soil aggregates rather than coarse-scale soil structure.

Importantly, the effects of invasive ungulates on soil properties varied substantially among sites (see also Wardle *et al.* 2001). Variability in nutrient concentrations and other physical properties are probable reasons for the variability among sites in the differences we observed in seedling growth between exclosure and control soils. Multiple regressions showed that a small but statistically significant proportion of the variation in seedling responses could be explained by variation among sites in the effects of ungulates on soil properties. For example, while across sites soil-mediated effects of ungulates did not significantly affect *K. excelsa* biomass overall (Fig. 2), ungulate effects on soil P concentrations (Bray extractable), soil total C and SOM explained significant amounts of the variation in *K. excelsa* biomass responses to these browsing herbivores. Similarly, soil-mediated effects of ungulates did not significantly affect *F. excorticata* biomass overall, but ungulate effects on soil pH explained a significant amount of variation in *F. excorticata* biomass responses. This suggests that changes in multiple soil properties, such as can be caused by invasive ungulates, are important determinants of plant performance, but these effects differ among sites (i.e. soil properties only sometimes differ between exclosures and control plots) and affect different plant species in different ways.

Among-site variation of ungulates on soil properties, and hence, plant performance, may have resulted from local or regional differences in ungulate densities. The invasive ungulates in New Zealand are not evenly distributed, and their densities depend on colonization and harvesting history, but also vary with topography and climate (King 2005). Soil-mediated effects for *F. excorticata* increased with increasing mean annual precipitation, and effects for *K. excelsa* decreased with increasing water deficit. Since soil moisture conditions in the greenhouse experiment were kept constant across sites, these water-related site effects must be indirect: 'dry' sites may, for example, be less attractive to ungulates. Among-site variation of ungulates on soil properties can also depend on site productivity (Coley *et al.* 1985; Milchunas & Lauenroth 1993) or, if effects are mediated through shifts in plant species dominance (Bellingham & Allan 2003; Mason *et al.* 2010), on forest type. However, regression analyses did not reveal significant effects of total standing biomass, or vegetation composition, on soil-mediated effects of ungulates on plant biomass. Taken together, these results show that site characteristics only had a limited capacity to explain among-site variation in how soil-mediated effects of invasive ungulates impact on plant performance.

Biotic and abiotic soil factors may interact in affecting plant performance in response to invasions of ungulates (Fig. 5), and we found evidence for our hypothesis that interactions between biotic and abiotic factors resulting from the presence of ungulates in turn affect the growth of tree seedlings. We expected that ungulates would impact on plant performance via shifts in AMF abundance (Eom, Wilson & Hartnett 2001; Gehring, Wolf & Theimer 2002), but this was not the case: infection of *M. ramiflorus* in Experiment 1 did not differ between soils from inside and outside the ungulate exclosures, and there were no effects of living soil inocula on seedling biomass or AMF root infection (Fig. 4a). These findings indicate that mycorrhizal infection potential did not differ between soils from inside and outside the exclosures. However, AMF root infection in Experiment 2 was higher in exclosure than control soils, regardless of the origin of the living soil inocula (exclosures vs. control plots). Moreover, across sites, effects of ungulates on seedling biomass were positively related to effects of ungulates on AMF root infection.

Taken together, these results suggest that effects of ungulates on plant performance could be mediated by AMF performance *as affected by* changes in soil properties. Soil compaction could be one possible explanation. Soil bulk density was higher outside than inside exclosures, and effects of ungulates on bulk density explained part of the among-site variation in AMF root infection. While we are unable to assess precisely how differences in soil bulk density as measured in the field translated to soil structure in our greenhouse experiments, it is recognized that increased soil bulk density (i.e. reduced microporosity) can negatively affect mycorrhizal growth and root infection and transfer of nutrients to the plant (Nadian *et al.* 1998; Rillig & Steinberg 2002; Rillig & Mummey 2006). Decreased mycorrhizal performance in compacted soils could have been caused by changes in soil aeration (Saif 1983), and/or by restriction of hyphal growth due to shifts in pore size distribution (Drew *et al.* 2003; Drew, Murray & Smith 2006). Moreover, soil compaction can differentially impact root colonization by different AMF species (Nadian *et al.* 1998). Further work is required to assess the degree to which effects of ungulates on AMF community structure may have played a role.

Finally, pathways by which ungulates affect plant performance may depend on plant species traits (Bardgett & Wardle 2003). While the limited number of species included in our study does not allow an in-depth analysis of species traits, it is worth mentioning that the patterns discussed above for *M. ramiflorus* are likely to apply to other arbuscular mycorrhizal species, but not to non-mycorrhizal species. For non-mycorrhizal species, the contribution of indirect and direct pathways by which ungulates affect plant growth would most likely include a more prominent role for the direct effects of physical and chemical soil properties (Fig. 5). However, we did not find evidence for any soil-mediated effects of ungulates on plant growth for the non-mycorrhizal species *K. excelsa*. Moreover, for *K. excelsa*, the production of cluster roots — an important adaptation for nutrient acquisition from nutrient-poor soils (Lambers *et al.* 2003) — did not differ between soils from inside and outside the ungulate exclosures.

Conclusions

Our study advances understanding of how invasive animals impact on forest ecosystems. Several studies have focussed on the impacts of invasive plants on plant-soil interactions and feedbacks (e.g. Stinson *et al.* 2006; Suding *et al.* 2013), but no such studies have been performed for invasive herbivores (Kardol & Wardle 2010). Using a network of long-term exclosure plots that represent considerable landscape-scale topographic and climatic variation, we show that the invasion of ungulates in New Zealand forests, from which they were absent until *c.* 200 years ago, can disrupt interactions between plants and soil organisms (see also Wood *et al.* 2011). This suggests that their impacts on forest dynamics are more complex than can be predicted based on selective browsing on palatable species alone. Determining the relative contribution of direct browsing effects and indirect soil-mediated effects, and whether or not these different effects interact to influence plant performance, is beyond the scope of our investigation.

An important next step would be to disentangle direct and indirect effects, for example by including an artificial grazing treatment (see, for example, Lagerström *et al.* 2011). There is also a need for further investigation of the mechanistic basis by which below-ground, cascading effects of invasive ungulates alter competitive interactions between palatable and unpalatable species, and may differentially affect plants that have contrasting nutrient acquisition strategies.

Finally, in many regions in New Zealand and elsewhere, much effort has been made in restoring ecosystems impacted by invasive ungulates, but often with limited success (e.g. Coomes *et al.* 2003; Bellingham *et al.* 2010). Restoration programmes often focus on eradication or reducing populations of invasive animals to low densities. However, reducing numbers of invasive animals alone may not be sufficient in creating conditions suitable for re-establishment of pre-invasion plant communities. Our results suggest that below-ground interventions that consider the abiotic and biotic soil legacies of invasive ungulates have considerable potential for facilitating ecological restoration of browser-invaded systems (see also Kardol and Wardle 2010).

Acknowledgements

We thank J. Buswell, K. Eaton, F. Hodge and S. Lucas for field assistance, and G. Rattray and F. Scheele for help in the laboratory. We thank C. Brigg for help with compiling data on site characteristics, S. Wiser for classifying the vegetation alliances, and the handling editor and two anonymous reviewers for helpful comments. This work was funded by a Wallenberg Scholars award to DAW, the New Zealand Department of Conservation's 'Wild Animal Control for Emissions Management' research programme (contract 4081) and Core funding for Crown Research Institutes from New Zealand's Ministry of Business, Innovation and Employment's Science and Innovation Group.

Data Accessibility

Biomass data, AMF data and cluster root data are available from the Dryad Digital Repository (Kardol *et al*. 2014).

References

Augustine, D.J. & McNaughton, S.J. (1998) Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. *The Journal of Wildlife Management*, **62**, 1165–1183.

Augustine, D.J., McNaughton, S.J. & Frank, D.A. (2003) Feedbacks between soil nutrients and large herbivores in a managed savanna ecosystem. *Ecological Applications*, **13**, 1325–1337.

Ayres, E., Heath, J., Possell, M., Black, H.I.J., Kerstiens, G. & Bardgett, R.D. (2004) Tree physiological responses to above-ground herbivory directly modify below-ground processes of soil carbon and nitrogen cycling. *Ecology Letters*, **7**, 469–479.

Bardgett, R.D. & Wardle, D.A. (2003) Herbivore-mediated linkages between aboveground and belowground communities. *Ecology*, **84**, 2258–2268.

Bardgett, R.D., Wardle, D.A. & Yeates, G.W. (1998) Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. *Soil Biology and Biochemistry*, **30**, 1867–1878.

Barrios-Garcia, M.N., Relva, M.A. & Kitzberger, T. (2012) Patterns of use and damage by exotic deer on native plant communities in northwestern Patagonia. *European Journal of Wildlife Research*, **58**, 137–146.

Beguin, J., Pothier, D. & Côté, S.D. (2011) Deer browsing and soil disturbance induce cascading effects on plant communities: a multilevel path analysis. *Ecological Applications*, **21**, 439–451.

Bellingham, P.J. & Allan, C.N. (2003) Forest regeneration and the influences of white-tailed deer (*Odocoileus virginianus*) in cool temperate New Zealand rain forests. *Forest Ecology and Management*, **175**, 71–86.

Bellingham, P.J., Wiser, S.K., Wright, A.E., Cameron, E.K. & Forester, L.J. (2010) Disperser communities and legacies of goat grazing determine forest succession on the remote Three Kings Islands, New Zealand. *Biological Conservation*, **143**, 926–938.

Bray, R.H. & Kurtz, L.T. (1945) Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, **59**, 39–45.

Caughley, G. (1970) Eruption of ungulate populations, with emphasis on Himalyan thar in New Zealand. *Ecology*, **51**, 53–72.

Coley, D., Bryant, J.P. & Chapin, F.S. (1985) Resource availability and plant antiherbivore defense. *Science*, **230**, 895–899.

Coomes, D.A., Allen, R.B., Forsyth, D.M. & Lee, W.G. (2003) Factors preventing the recovery of New Zealand forests following control of invasive Deer. *Conservation Biology*, **17**, 450–459.

Côté, S.D., Rooney, T.P., Tremblay, J.-P., Dussault, C. & Waller, D.M. (2004) Ecological impacts of deer overabundance. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 113–147.

Crawley, M.J. (2007) *The R Book*. Wiley, Chichester.

Díaz, S., Lavorel, S., McIntyre, S., Falczuk, V., Casanoves, F., Milchunas, D.G. *et al.* (2007) Plant trait responses to grazing – a global synthesis. *Global Change Biology*, **13**, 313–341.

Diez, J.M., Dickie, I.A., Edwards, G., Hulme, P.E., Sullivan, J.J. & Duncan, R.P. (2010) Negative soil feedbacks accumulate over time for non-native plant species. *Ecology Letters*, **13**, 803–809.

Drew, E.A., Murray, R.S. & Smith, S.E. (2006) Functional diversity of external hyphae of AM fungi: ability to colonise new hosts is influenced by fungal species, distance and soil conditions. *Applied Soil Ecology*, **32**, 350–365.

Drew, E.A., Murray, R.S., Smith, S.E. & Jakobsen, I. (2003) Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. *Plant and Soil*, **251**, 105–114.

Duncan, K. & Holdaway, R. (1989) Footprint pressure and locomotion of moas and ungulates and their effects on the New-Zealand indigenous biota through trampling. *New Zealand Journal of Ecology*, **12**, 97–101.

Eom, A.H., Wilson, G.W.T. & Hartnett, D.C. (2001) Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. *Mycologia*, **92**, 233–242.

Frank, D.A. & Groffman, P.M. (1998) Ungulate vs. landscape control of soil C and N processes in grasslands of Yellowstone National Park. *Ecology*, **79**, 2229–2241.

Gass, T.M. & Binkley, D. (2011) Soil nutrient losses in an altered ecosystem are associated with native ungulate grazing. *Journal of Applied Ecology*, **48**, 952–960.

Gehring, C.A., Wolf, J.E. & Theimer, T.C. (2002) Terrestrial vertebrates promote arbuscular mycorrhizal fungal diversity and inoculum potential in a rain forest soil. *Ecology Letters*, **5**, 540–548.

Grime, J.P., Cornelissen, J.H.C., Thompson, K. & Hodgson, J.G. (1996) Evidence of a causal connection between anti-herbivore defense and the decomposition rate of leaves. *Oikos*, **77**, 489–494.

Habeck, J.R. (1960) Winter deer activity in the white cedar swamps of Northern Wisconsin. *Ecology*, **41**, 327–333.

Hamilton, E.W. & Frank, D.A. (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology*, **82**, 2397–2402.

Hamilton, E.W., Frank, D.A., Hinchey, P.M. & Murray, T.R. (2008) Defoliation induces root exudation and triggers positive rhizosphere feedbacks in a temperate grassland. *Soil Biology and Biochemistry*, **40**, 2865–2873.

Harrison, K. & Bardgett, R.D. (2004) Browsing by red deer negatively impacts on soil nitrogen availability in regenerating native forest. *Soil Biology and Biochemistry*, **36**, 115–126.

Heckel, C.D., Bourg, N.A., McShea, W.J. & Kalisz, S. (2010) Nonconsumptive effects of a generalist ungulate herbivore drive decline of unpalatable forest herbs. *Ecology*, **91**, 319–326.

Hurst, J.M. & Allen, R.B. (2007) *A permanent plot method for monitoring indigenous forests – Field Protocols*. Landcare Research Manaaki Whenua, Lincoln, NZ. http://nvs.landcareresearch.co.nz/html/PermanentPlot_Expanded-Manual.pdf

Husheer, S.W., Allen, R.B. & Robertson, A.W. (2006) Suppression of regeneration in New Zealand mountain beech forests is dependent on species of introduced deer. *Biological Invasions*, **8**, 823–834.

Jackson, M.L. (1958) *Soil Chemical Analysis*. Prentice Hall, Englewood Cliffs, NJ.

Kardol, P., Dickie, I.A., St. John, M.G., Husheer, S.W., Bonner, K.I., Bellingham, P.J. & Wardle, D.A. (2014) Data from: Soil-mediated effects of invasive ungulates on native tree seedlings. *Dryad Digital Repository*, doi: 10.5061/dryad.98c81.

Kardol, P. & Wardle, D.A. (2010) How understanding aboveground–belowground linkages can assist restoration ecology. *Trends in Ecology and Evolution*, **25**, 670–679.

Kardol, P., Cornips, N.J., van Kempen, M.M.L., Bakx-Schotman, J.M.T. & van der Putten, W.H. (2007) Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs*, **77**, 147–162.

King, C.M. (2005) *The Handbook of New Zealand Mammals*, 2nd edn. Oxford University Press, South Melbourne.

Lagerström, A., Bellingham, P.J., Bonner, K.I. & Wardle, D.A. (2011) The effect of simulated herbivory on growth and nutrient status of focal and neighbouring early successional woody plant species. *Oikos*, **120**, 1380–1392.

Lambers, H., Cramer, M.D., Shane, M.W., Wouterlood, M. & Veneklaas, E.J. (2003) Structure and functioning of cluster roots and plant responses to phosphate deficiency. *Plant and Soil*, **248**, IX–XIX.

Mason, N.W.H., Peltzer, D.A., Richardson, S.J., Bellingham, P.J. & Allen, R.B. (2010) Stand development moderates effects of ungulate exclusion on foliar traits in the forests of New Zealand. *Journal of Ecology*, **98**, 1422–1433.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. & Swan, J.A. (1990) A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. *New Phytologist*, **115**, 495–501.

Milchunas, D.G. & Lauenroth, W.K. (1993) Quantitative effects of grazing on vegetation and soils over a global range of environments. *Ecological Monographs*, **63**, 328–366.

Mulder, C.P.H., Bazeley-White, E., Dimitrakopoulos, P.G., Hector, A., Scherer-Lorenzen, M. & Schmid, B. (2004) Species evenness and productivity in experimental plant communities. *Oikos*, **107**, 50–63.

Nadian, H., Smith, S.E., Alston, A.M., Murray, R.S. & Siebert, B.D. (1998) Effects of soil compaction on phosphorus uptake and growth of *Trifolium subterraneum* colonized by four species of vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, **139**, 155–165.

Nuñez, M., Bailey, J.K. & Schweitzer, J.A. (2010) Population, community and ecosystem effects of exotic herbivores: a growing global concern. *Biological Invasions*, **12**, 297–301.

Pastor, J., Dewey, B., Naiman, R.J., McInnes, P.F. & Cohen, Y. (1993) Moose browsing and soil fertility in the boreal forests of Isle Royale National Park. *Ecology*, **74**, 467–480.

Rillig, M.C. & Mummey, D.L. (2006) Mycorrhizas and soil structure. *New Phytologist*, **171**, 41–53.

Rillig, M.C. & Steinberg, P.D. (2002) Glomalin production by an arbuscular mycorrhizal fungus: a mechanism of habitat modification? *Soil Biology and Biochemistry*, **34**, 1371–1374.

Saif, S.R. (1983) The influence of soil aeration on the efficiency of vesicular-arbuscular mycorrhizas II. Effects of soil oxygen on growth and mineral uptake in *Eupatorium odoratum* L., *Sorghum bicolor* (L.) Moench and *Guizotia abyssinica* (L.f.) Cass. inoculated with vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, **95**, 405–417.

Shane, M.W. & Lambers, H. (2005) Cluster roots: a curiosity in context. *Plant and Soil*, **274**, 101–125.

Sørensen, L.I., Kytoviita, M.-M., Olofsson, J. & Mikola, J. (2008) Soil feedback on plant growth in a sub-arctic grassland as a result of repeated defoliation. *Soil Biology and Biochemistry*, **40**, 2891–2897.

St. John, M.G., Bellingham, P.J., Walker, L.R., Orwin, K.H., Bonner, K.I., Dickie, I.A., Morse, C.W., Yeates, G.W. & Wardle, D.A. (2012) Loss of a dominant nitrogen-fixing shrub in primary succession: consequences for plant and below-ground communities. *Journal of Ecology*, **100**, 1074–1084.

Stevenson, B.A. & St. John, M.G. (2009) *Soil sampling protocol for WACEM*. Landcare Research Contract Report LC0809/077.

Stinson, K.A., Campbell, S.A., Powell, J.R., Wolfe, B.E., Callaway, R.M., Thelen, G.C., Hallett, S.G., Prati, D. & Klironomos, J.N. (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PloS Biology*, **4**, 727–731.

Suding, K.N., Harpole, W.S., Fukami, T., Kulmatiski, A., MacDougall, A.S., Stein, C. & van der Putten, W.H. (2013) Consequences of plant–soil feedbacks in invasion. *Journal of Ecology*, **101**, 298–308.

Van der Wal, R., Bardgett, R.D., Harrison, K.A. & Stien, A. (2004) Vertebrate herbivores and ecosystem control: cascading effects of faeces on tundra ecosystems. *Ecography*, **27**, 242–252.

Vazquez, D.P. (2002) Multiple effects of introduced mammalian herbivores in a temperate forest. *Biological Invasions*, **4**, 175–191.

Wardle, D.A. & Bardgett, R.D. (2004) Human-induced changes in large herbivorous mammal density: the consequences for decomposers. *Frontiers in Ecology and the Environment*, **2**, 145–153.

Wardle, D.A., Bonner, K.I. & Barker, G.M. (2002) Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. *Functional Ecology*, **16**, 585–595.

Wardle, D.A., Barker, G.M., Yeates, G.W., Bonner, K.I. & Ghani, A. (2001) Introduced browsing mammals in New Zealand natural forests: aboveground and belowground consequences. *Ecological Monographs*, **71**, 587–614.

Wardle, D.A., Bellingham, P.J., Fukami, T. & Bonner, K.I. (2012) Soil-mediated indirect impacts of an invasive predator on plant growth. *Biology Letters*, **8**, 574–577.

Wiser, S.K. & De Cáceras, M. (2013) Updating vegetation classifications: an example with New Zealand's woody vegetation. *Journal of Vegetation Science*, **24**, 80–93.

Wiser, S.K., Hurst, J.M., Wright, E.F. & Allen, R.B. (2011) New Zealand's forest and shrubland communities: a quantitative classification based on a nationally representative plot network. *Applied Vegetation Science*, **14**, 506–523.

Wood, J.R., Wilmshurst, J.M., Worthy, T.H. & Cooper, A. (2011) *Sporormiella* as a proxy for non-mammalian herbivores in island ecosystems. *Quarternary Science Reviews*, **30**, 915–920.

Received 10 September 2013; accepted 18 February 2014
Handling Editor: Dali Guo

Supporting Information

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

Table S1. Site descriptions.

Table S2. Trait characteristics for six New Zealand tree species used in Experiment 1.

Table S3. Summary of plant species and exclosure effects on total seedling biomass in Experiment 1.

Table S4. Summary of exclosure effects on seedling root biomass, shoot biomass and root–shoot ratio in Experiment 1.

Table S5. Summary of plant species and exclosure effects on seedling root biomass, shoot biomass and root:shoot ratio in Experiment 1.

Table S6. Summary of exclosure effects on soil abiotic properties.

Table S7. Results from multiple regressions testing relationships between site characteristics and the effects of ungulates on total seedling biomass.

Table S8. Summary of model comparisons testing root biomass, shoot biomass and root–shoot ratio responses in Experiment 2.