

# Cascading effects of long-term land-use changes on plant traits and ecosystem functioning

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**Abstract.** There is much concern that the functioning of ecosystems will be affected by human-induced changes in biodiversity, of which land-use change is the most important driver. However, changes in biodiversity may be only one of many pathways through which land use alters ecosystem functioning, and its importance relative to other pathways remains unclear. In particular, although biodiversity–ecosystem function research has focused primarily on grasslands, the increases in agricultural inputs (e.g., fertilization, irrigation) and grazing pressure that drive change in grasslands worldwide have been largely ignored. Here we show that long-term (27-year) manipulations of soil resource availability and sheep grazing intensity caused marked, consistent shifts in grassland plant functional composition and diversity, with cascading (i.e., causal chains of) direct, indirect, and interactive effects on multiple ecosystem functions. Resource availability exerted dominant control over above-ground net primary production (ANPP), both directly and indirectly via shifts in plant functional composition. Importantly, the effects of plant functional diversity and grazing intensity on ANPP shifted from negative to positive as agricultural inputs increased, providing strong evidence that soil resource availability modulates the impacts of plant diversity and herbivory on primary production. These changes in turn altered litter decomposition and, ultimately, soil carbon sequestration, highlighting the relevance of ANPP as a key integrator of ecosystem functioning. Our study reveals how human alterations of bottom-up (resources) and top-down (herbivory) forces together interact to control the functioning of grazing systems, the most extensive land use on Earth.

**Key words:** *biodiversity; carbon sequestration; ecosystem services; functional diversity; grasslands; grazing intensity; litter decomposition; long-term experiment; Mackenzie Basin, South Island, New Zealand; primary production; resource availability; structural equation modeling.*

## INTRODUCTION

Concerns over global biodiversity decline from human activities (Pimm and Raven 2000) have triggered a vast body of research exploring its potential impacts on ecosystem functioning: the rates of processes that control the flow of energy and matter across ecosystem compartments (Chapin et al. 2000, Hooper et al. 2005). Of all global drivers of biodiversity loss, land-use change is considered to be the most important (Sala et al. 2000). However, because land-use change implies fundamental alterations to the bottom-up (e.g., resource availability) and top-down (e.g., biomass removal) forces that both influence biodiversity and directly control ecosystem processes (Foley et al. 2005), biodiversity loss may be only one of many ways through which changes in land use alter ecosystem functioning (Chapin et al. 2000,

Hooper et al. 2005, Srivastava and Vellend 2005, Hillebrand and Matthiessen 2009). As a result, we must move beyond simply asking whether biodiversity matters to the functioning of ecosystems, to instead ask how it matters, and by how much relative to other drivers (Hooper et al. 2005, Srivastava and Vellend 2005, Díaz et al. 2007, Grace et al. 2007, Hillebrand and Matthiessen 2009).

Although grasslands have been used widely in biodiversity–ecosystem function experiments (Hooper et al. 2005, Balvanera et al. 2006), explicitly considering the increases in agricultural inputs (e.g., fertilization, irrigation) and grazing pressure that drive change in grasslands worldwide (Bouwman et al. 2005) would yield further insights into how land-use changes alter their functioning. In grasslands, soil resource availability can control ecosystem functioning either directly (Burke et al. 1997, Díaz et al. 2007, Lambers et al. 2008) or indirectly via shifts in plant traits (Díaz et al. 2007, Quétier et al. 2007; see Appendix A). Similarly, grazing intensity can exert direct control over ecosystem processes (McNaughton et al. 1997) or indirect control

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through shifts in plant composition (Bardgett and Wardle 2003, Bagchi and Ritchie 2010; see Appendix A).

Soil resource availability and disturbances such as grazing are also widely thought to be key controllers of plant diversity (Grime 1973, Huston 1994, Collins et al. 1998). Importantly, the extent to which changes in plant diversity translate into changes in ecosystem functioning is expected to depend on environmental context (Wardle and Zackrisson 2005), particularly soil resource availability (Fridley 2002, 2003; see Appendix A). Likewise, the direction and strength of grazing impacts on vegetation and ecosystem processes also have been predicted to depend on soil resource availability (Olff and Ritchie 1998, Bardgett and Wardle 2003; see Appendix A). As a result, calls have been made for “next-generation” biodiversity–ecosystem function experiments that explicitly consider realistic drivers of change in biodiversity (Srivastava and Vellend 2005, Hillebrand and Matthiessen 2009), as well as for comprehensive experiments that explore the direct and indirect herbivore impacts on aboveground and belowground processes in ecosystems of varying soil resource availability (Bardgett and Wardle 2003). Our study addresses both of these calls.

To do this, we used long-term (27-year), realistic manipulations of soil resource availability and grazing intensity in experimental grasslands (Scott 1999). The experiment consisted of the initial sowing of a common plant species pool (25 species) into resident grassland vegetation (30 large  $8 \times 50$  m plots) in 1982, with annual manipulations of soil resource availability (five fertilization levels, the lowest receiving no fertilizer, and the highest also being irrigated to represent realistic high-intensity agriculture) and sheep grazing intensity (three levels, leading to different proportions of ANPP grazed).

We employed a response–effect trait framework, which is recommended for quantifying the functional consequences of changes in biodiversity (Chapin et al. 2000, Hooper et al. 2005, Díaz et al. 2007, Hillebrand and Matthiessen 2009). We used specific leaf area (community-weighted leaf area to dry mass ratio) as a marker of plant functional composition (Garnier et al. 2004) because specific leaf area can respond strongly to shifts in soil resource availability or grazing in grasslands (Quétiér et al. 2007) and, in turn, can influence biogeochemical processes (Garnier et al. 2004, Díaz et al. 2007, Quétiér et al. 2007). We also estimated plant functional diversity using a broader range of plant morphological and chemical traits. Functional diversity is widely thought to provide a clearer link than species richness to complementary resource use (Hooper et al. 2005, Díaz et al. 2007, Hillebrand and Matthiessen 2009), one of the key mechanisms proposed to explain positive effects of biodiversity on ecosystem functioning (Hooper et al. 2005). Although functional composition and diversity were not directly manipulated, the long-term experimental treatments generated strong, consistent differences in plant composition and diversity (Scott

2001), which we combined with statistical controls, using structural equation modeling (Grace et al. 2007, Shipley 2009). This approach provides powerful insights into the importance of diversity effects on ecosystem functioning relative to other factors, while maintaining community changes that fall within natural bounds of relevance (Srivastava and Vellend 2005, Grace et al. 2007).

## MATERIALS AND METHODS

### *Study area and site*

The study was conducted on the AgResearch trial site at Mount John, west of Lake Tekapo in the Mackenzie Basin of New Zealand's South Island ( $43^{\circ}59'$  S,  $170^{\circ}27'$  E, 820 m above sea level; see Plate 1). The climate is semi-continental with an average of 1772 degree-days above  $5^{\circ}\text{C}$  and a mean annual temperature of  $8.7^{\circ}\text{C}$ , with  $14.9^{\circ}\text{C}$  in January (warmest) and  $1.7^{\circ}\text{C}$  in July (coldest). Mean annual rainfall is 601 mm and is uniformly distributed throughout the year. The average annual moisture deficit is 445 mm. Soils are humose, orthic brown (Hewitt 1998), 45–90 cm deep, and were developed from greywacke and argillite rock till material. The dominant vegetation type prior to human settlement is likely to have been short-tussock grassland with a variable woody component, probably near the tall tussock (*Chionochloa* spp.)/short tussock (*Festuca novae-zelandiae* Hack. Cockayne) transition (McGlone 2001). Early human occupation began around 700–800 years ago, and has been linked with increased fire frequency (McGlone 2001). Extensive grazing by sheep was introduced to the area in the 1850–1860s and remains the most prevalent land use today.

### *Experimental design*

The experiment is described in detail elsewhere (Scott 1999) and is only summarized here. In 1982, a homogeneous mixture of 25 grass and legume pasture species was over-sown, using a rotary hoe drill, within a 3-ha area of depleted fescue-tussock (*F. novae-zelandiae*) grassland dominated by the exotic mouse-ear hawkweed (*Hieracium pilosella* L.). The trial followed a split-plot design consisting of two spatial replications (blocks), each split into five whole plots receiving one of the following five nominal fertilizer treatments: 0, 50, 100, 250, and  $500 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  of sulfur-fortified superphosphate (i.e., a P/S fertilizer), which is typical of Australasian legume-based developed pastoral systems. The actual mean P and S rates applied over the first 10 years of the experiment, in terms of applied P+S, were: 0+0, 4.1+17.6, 8.9+26.0, 22.7+54.5, and 46.8+114.8  $\text{kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  for the 0, 50, 100, 250 and  $500 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  treatments, respectively (Scott 1999). The whole plots receiving  $500 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  were also irrigated fortnightly from November to May of each year to simulate the highest intensity agriculture in the region. Fertilizer was applied each year for the first 20 years of the experiment.

Each whole plot was further split into six  $8 \times 50$  m subplots (hereafter simply referred to as “plots”)

TABLE 1. Description of variables used in the analyses.

Variable	Units	Range
Soil resource availability	unitless	1–5
Grazing intensity (proportion of ANPP grazed; 18-month period)	unitless	0.3–1.1†
Specific leaf area (community-weighted mean)	m <sup>2</sup> /kg	12.3–20.8
Functional diversity (multivariate dispersion)	unitless	0.05–0.36
Aboveground net primary production (18-month period)	Mg dry matter (DM)/ha	1.7–34.3
Belowground net primary production (18-month period)	Mg dry matter (DM)/ha	3.6–9.5
Soil carbon (0–20 cm)	Mg C/ha	57.4–110.9
Soil carbon (60–80 cm)	Mg C/ha	12.5–45.0
Litter decomposition rate ( $k \times 10^3$ )	unitless	0.97–8.44

† Values above 1 could be obtained because the measurement period started near peak standing biomass, but ended directly after all plots were grazed before the winter resting period (see *Methods*).

corresponding to a two-way factorial design involving sheep grazing intensity (lax, moderate, and hard) and stocking type (mob vs. sustained). In mob-grazing plots, a larger number of sheep (with actual numbers depending on available feed-on-offer of the moderate plot) were introduced to plots for 3–4 days, whereas sustained-grazing plots received a lower number of sheep for a longer period (e.g., several weeks). However, only plots corresponding to the mob-stocking type were considered in the present study.

Plots were always grazed in groups of three, corresponding to the three mob-stocked grazing intensity levels per whole plot. For each grazing event (i.e., when vegetation had reached ~30 cm in height), sheep numbers were adjusted based on available feed-on-offer of the moderate grazing treatment, using expert knowledge (D. Scott, *personal communication*). The duration of grazing was the same for all three plots, but was adjusted based on residual bulk of the moderate grazing treatment (i.e., height of 1–2 cm). Grazing intensity levels corresponded to a 1:2:4 sheep-grazing days ratio in years 2–4 of the experiment and 2:3:4 in subsequent years; a 2:3:4 ratio means that if 30 sheep were used in the moderate treatment, then the low treatment was stocked with 20 sheep, while the high treatment was stocked with 40. Grazing treatments occurred in the period November–May each year. Depending on the fertilizer level, there were 4–9 grazing cycles during our sampling period (October 2007–April 2009).

#### Vegetation sampling

Sampling of all vascular plant species present within each plot was undertaken in November 2007 and 2008 (Laliberté et al. 2010). Twenty 1 × 1 m quadrats were positioned randomly along two longitudinal transects in each plot. Transects were 3 m apart from each other and 2 m from the closest fence. Cover of all vascular plant species present in each 1 × 1 m quadrat was recorded using a seven-point semiquantitative scale (1, ≤ 0.1%; 2, 0.1–0.9%; 3, 1–5%; 4, 6–25%; 5, 26–50%; 6, 51–75%; 7,

76–100%). Mean percent cover per species per plot was calculated by taking the median of the cover class for each species in each of the 20 quadrats, then averaging across these quadrats. Species present within the entire plot but not in the 20 individual 1 × 1 m quadrats were assigned the lowest possible cover value. Percent cover data per plot were transformed to relative abundances. Relative abundance data from 2007 and 2008 were averaged.

#### Plant functional traits and functional diversity

We selected a set of leaf traits that have been identified as suitable to predict plant species responses to environmental change and effects on ecosystem processes in herbaceous communities, while still being possible to measure across a wide range of species (Cornelissen et al. 2003, Garnier et al. 2007). Specific leaf area (SLA) and leaf dry matter content (LDMC) were measured on at least 10 individuals per plant species (spread among the five fertilizer treatments), following a standardized protocol (Garnier et al. 2001). A composite sample of 10 individuals per species (spread among the five fertilizer treatments) was used to estimate leaf carbon concentration (LCC), nitrogen concentration (LNC), phosphorus concentration (LPC), and sulfur concentration (LSC). These samples were oven-dried at 60°C, ground, and then sent to a commercial laboratory for analysis (Hill Laboratories, Hamilton, New Zealand). Because leaf nutrient concentrations can vary along resource availability gradients, we used individual samples from each fertilizer level for the six species that together accounted for >80% of total cover among all plots (Garnier et al. 2007). Field-measured traits were measured on all vascular plant species found in all experimental plots in November 2007, just prior to the start of the annual grazing treatments.

For our analyses, we focused on the community-weighted mean of SLA (Table 1) as a marker of functional composition (Garnier et al. 2004). Specific leaf area, which expresses the area available for light interception per unit leaf dry mass, is expected to

respond strongly to shifts in soil resource availability and grazing intensity (Lavorel and Garnier 2002, Díaz et al. 2007, Quétier et al. 2007). It is also an important component of plant relative growth rate (Lambers et al. 2008) and has been shown to correlate with primary production and other biogeochemical processes, including litter decomposition and ANPP (Garnier et al. 2004). In our study, SLA was strongly negatively correlated with leaf C:N ( $r = -0.89$ ,  $P < 0.0001$ ), which is a good estimate of litter quality for herbaceous species and thus influences litter decomposition rate (Lambers et al. 2008). Although SLA and leaf traits in general do not directly capture belowground strategies (De Deyn et al. 2008), previous work in grasslands of varying fertility within our study area observed consistent coupling between leaf and root morphological and chemical traits (Craine and Lee 2003), suggesting that our leaf traits may act as proxies for belowground strategies.

We estimated plant functional diversity (FD) using SLA, LDMC, LNC, LSC, and LPC. We used functional dispersion as our abundance-weighted FD index (Table 1), which is simply the multivariate analogue of the weighted mean absolute deviation (Laliberté and Legendre 2010). Functional diversity metrics were computed using the “FD” package (Laliberté and Legendre 2010) in the R environment (R Development Core Team 2011).

#### *Aboveground net primary production*

Aboveground net primary production (ANPP; Table 1) was estimated during an 18-month period (October 2007–April 2009), using periodic measurements with a capacitance probe (GrassMaster II, Novel Ways, Hamilton, New Zealand) that was calibrated against oven-dried live biomass harvests from 100 circular quadrats taken across all plots in October 2007 ( $R^2 = 0.737$ ,  $P < 0.0001$ ). Each sampling event consisted of 100 capacitance probe measurements per plot (evenly spread across four longitudinal transects), which were then used to estimate the mean live standing biomass. Capacitance probe measurements were always made directly prior to and after each grazing event in each plot, thus allowing us to quantify the amount of primary production consumed or trampled by sheep over that period. Periodic routine measurements (about once a month) were also made between grazing events. Measurements were made when vegetation was dry to prevent overestimation of plant live biomass. All increases in live biomass between two consecutive measurements were summed over the entire sampling period to calculate ANPP (Lauenroth 2000). Our approach for measuring ANPP avoids the two major methodological problems that previously have complicated the study of grazing impacts on ANPP in grazing systems and the importance of compensatory plant growth in particular (McNaughton 1983). These problems are that (1) grazing animals must be able to remove vegetation biomass during the period of estimation, and

that (2) consumption by grazing animals must be quantified and taken into account (McNaughton et al. 1996).

#### *Belowground net primary production*

Belowground net primary production (BNPP; Table 1) was measured using root ingrowth cores. Four soil cores per plot (8 cm diameter  $\times$  30 cm deep), randomly positioned along a central longitudinal transect, were removed in October 2007. The resulting holes were immediately filled with root-free soil enclosed in 1-mm mesh nylon bags. Root cores were retrieved in May 2008, cut into three sections corresponding to three depths (0–10 cm, 10–20 cm, and 20–30 cm) and each section was washed through a 1-mm sieve to separate soil from roots, which were then oven-dried and weighed. The same procedure, again with four root ingrowth cores per plot, was repeated for the July 2008–April 2009 period. For each harvested root ingrowth core, we summed all three depth sections and then averaged values from all four cores per plot for each sampling period. Finally, values from the two sampling periods were summed.

#### *Litter decomposition*

Litter decomposition (Table 1) was measured following a standardized procedure (Garnier et al. 2007). In each plot, freshly senesced plant litter was collected in March 2008 from 10  $1 \times 1$  m quadrats randomly positioned along a central longitudinal transect. Live biomass, obvious seeds, sheep feces, and highly decomposed material were discarded. Litter was air-dried for two weeks, cut into  $\sim 5$ -cm segments, and mixed to yield a “community litter” (Aerts et al. 2003). Nylon litter bags ( $1 \times 1$  mm mesh size) were filled with  $\sim 2$ -g portions of dry litter. Litter subsamples (10 per plot) were oven-dried for air-dry mass to oven-dry mass conversions.

Litter was field-incubated back into the plots from where it came. Live plants and stubble were cut to the soil surface with a spade in four randomly positioned areas along a central longitudinal transect, within which litter bags were incubated. Four litter bags per plot and harvest date (after 1, 3, 6, 12, and 18 months of incubation) were used, for a total of 20 litter bags per plot (600 bags in total). Rabbit fencing was laid on top of the litter bags to ensure good contact with the soil and to prevent bags from being blown away by the wind. Litter bags were also enclosed within sheep enclosures to prevent them from being trampled and damaged. Bags were checked periodically to remove any live plant ingrowth (mostly grass and clover leaves, which were carefully removed). At each harvest date, four litter bags were collected from each plot, oven-dried, and weighed.

In addition to incubation within experimental plots, community litter was also decomposed under standard conditions in a homogeneous section of the study site, in order to assess the effects of litter quality alone. Four litter bags (i.e., replicates) per plot and harvest date (1,

3, 6, 12, and 18 months of incubation) were used, for a total of 600 bags. The same procedure as previously described was used for installation and maintenance. In all cases, we used the negative exponential single-pool decomposition model (Olson 1963) to estimate litter decomposition rate (Appendix B).

#### *Soil carbon*

In each plot, four soil samples from each of two depths (0–20 cm and 60–80 cm) were collected at random locations along a central longitudinal transect (September 2008). Because getting soil samples from the 60–80 cm depth was difficult, and because we needed to minimize physical damage to experimental plots, we could not take more than four samples per plot. A composite soil sample per depth was created from these four samples and analyzed for total C. Total C data were corrected with bulk density (BD) data to estimate soil C storage (SC), and results are reported as Mg C/ha (for 0–20 cm and 60–80 cm depths; Table 1):

$$SC_{0-20\text{ cm}} = 20 \times \%C_{0-20\text{ cm}} \times BD$$

$$SC_{60-80\text{ cm}} = 20 \times \%C_{60-80\text{ cm}} \times BD.$$

#### *Statistical analyses*

We used generalized multilevel path models (Shipley 2009) to test multivariate hypotheses involving the relative influence of soil resource availability and grazing intensity on plant functional composition and diversity and on ecosystem processes (Appendix A). Standard structural equation modeling (SEM) approaches based on comparisons between observed and predicted covariance matrices could not be used because the hierarchical nature of the experimental design violated the assumption of independence among observations. Besides allowing us to consider the hierarchical nature of the design, another benefit of generalized multilevel path models is that they offer a flexible way to take into account nonlinear relationships and interactions among variables (Shipley 2009). Details of these analyses are given in Appendix C.

### RESULTS

The long-term manipulations of soil resource availability and grazing intensity caused marked shifts in plant functional composition, as measured via community-weighted specific leaf area (Fig. 1). Specific leaf area increased sharply from the lowest to the second lowest resource availability level, but then varied little with further increases in resource availability (Appendix D).

These changes in plant functional composition toward greater specific leaf area were associated with strong reductions in plant functional diversity (Fig. 1). In contrast, grazing intensity directly increased plant functional diversity, but the strength of this effect depended on soil resource availability (Fig. 1). Indeed,

grazing intensity had little influence on functional diversity at the lowest resource availability level, but its effect became increasingly positive as resource availability increased (Fig. 2a).

Resource availability had strong direct positive effects on ANPP (holding grazing intensity and functional diversity at their mean values), as well as indirect positive effects via shifts in specific leaf area (Fig. 1). Strikingly, the impacts of functional diversity and grazing intensity on ANPP switched from negative to positive as soil resources increased (Figs. 1 and 2b, c). In contrast to ANPP, BNPP was not influenced by soil resource availability, grazing intensity, or plant functional diversity, and was decoupled from ANPP itself (Fig. 1). On the other hand, resource availability, grazing intensity, and their associated impacts on plant functional composition and diversity had cascading effects on litter decomposition, primarily via their effects on ANPP (Fig. 1).

Resource availability had a dominant, nonlinear direct effect on the amount of carbon stored in the soil surface (0–20 cm depth; Fig. 1), whereby soil carbon increased progressively with fertilizer rate but then dropped sharply at the highest resource availability level (Appendix E). In addition, ANPP, specific leaf area, grazing intensity, and BNPP all had direct positive effects on surface soil carbon (Fig. 1). Surface soil carbon, in turn, directly increased the amount of soil carbon sequestered in deeper soil layers (60–80 cm), whereas resource availability strongly decreased deep soil carbon (Fig. 1).

### DISCUSSION

Our results showed that long-term (27-year) changes in soil resource availability and grazing intensity in experimental grasslands caused shifts in plant functional composition and functional diversity, with cascading effects on multiple ecosystem functions. Resource availability exerted dominant control over ecosystem functioning, both through direct and indirect effects and by altering the impacts of other drivers.

Direct positive effects of soil resource availability on ANPP were probably caused by greater plant resource uptake (Burke et al. 1997, Lambers et al. 2008). Increases in soil resource availability were also associated with changes in plant functional composition toward greater specific leaf area, as predicted (Díaz et al. 2007, Quétier et al. 2007). Specific leaf area is tightly linked to the leaf economics spectrum that separates slow-growing from fast-growing species (Wright et al. 2004) and correlates negatively with leaf life span (Reich et al. 1992). Therefore, these results are consistent with the view that leaf attributes that reduce nutrient losses hold a long-term advantage under nutrient limitation, whereas those associated with a rapid growth rate become dominant under high fertility (Chapin 1980). Increases in specific leaf area, in turn, were associated



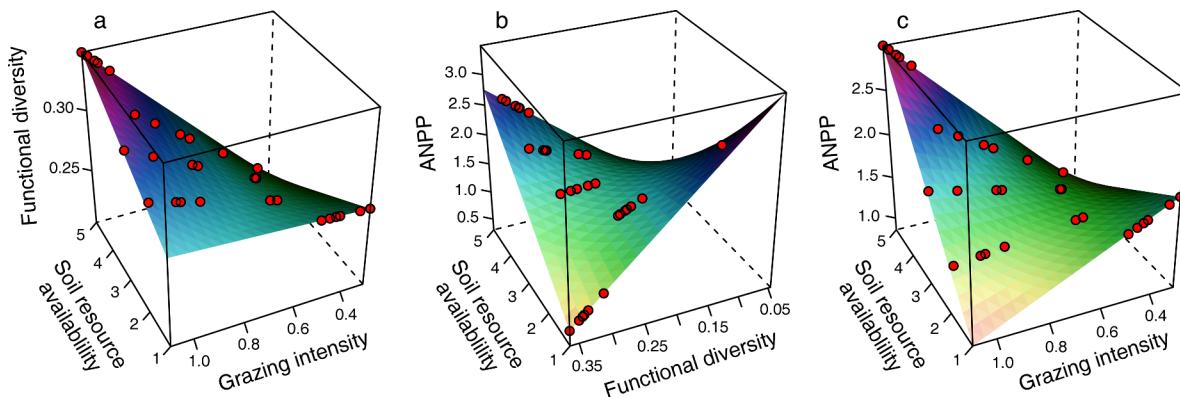


FIG. 2. Interactive effects on plant functional diversity and ANPP. In each panel, the surface represents the population fitted values for the two terms involved in the interaction, holding all other fixed terms in the model constant at their mean values. The ranges of the bottom axes ( $x$  and  $y$ ) were determined from the range of the observed data. Red dots show the population fitted values associated with individual plots. ANPP data were ln-transformed.

inherently slower-growing species (Aerts 1999). In contrast, higher grazing intensity was associated with greater functional diversity, with this effect strengthening as resource availability increased, possibly because grazing can suppress plant competitive dominance (Grime 1973, Collins et al. 1998). Surprisingly, higher plant functional diversity was associated with lower ANPP under lower soil resource availability, but this relationship became increasingly positive with greater soil resources, its direction ultimately reversing. These results, which agree with those from a short-term grassland experiment in which plant species richness and soil resource availability were independently manipulated (Fridley 2002), highlight the importance of soil resources for ecosystem functioning and suggest that positive effects of plant functional diversity on production may only become important when resource availability is high.

Fridley (2002) suggested two potential mechanisms to explain the stronger positive effects of diversity under higher soil resource availability. First, complementary resource use may be stronger when resources are plentiful, allowing functionally diverse mixtures to take up a greater amount of resources than less diverse ones. A second possibility is that facilitative interactions between neighboring plants may become more important under higher fertility. In a follow-up study, Fridley (2003) found that higher fertility promoted overyielding of diverse plant mixtures relative to monocultures, and that this was due to light partitioning rather than facilitation. In our study, we cannot distinguish between these two possible mechanisms to explain why plant functional diversity increased production at high, but not low, soil resource availability.

Strikingly, impacts of grazing intensity on ANPP also shifted from negative to positive with increasing soil resources. The tenet that impacts of grazing by large herbivores on ecosystem processes depend on soil resource availability has been hypothesized (Bardgett

and Wardle 2003) and recently supported by field studies along natural resource gradients (Augustine and McNaughton 2007), yet until now strong experimental tests have remained elusive. Although the mechanisms underlying this interactive effect are unresolved, above-ground–belowground feedbacks are likely to be involved (Bardgett and Wardle 2003). One such feedback pathway could be that under greater soil resource availability, increases in root exudation through grazing-induced defoliation stimulated microbial decomposition and nitrogen mineralization (Hamilton et al. 2008), thereby promoting plant growth. Conversely, under low soil resource availability, such beneficial effects of grazing can be outweighed by other detrimental impacts. For example, mycorrhizal associations play significant roles in plant mineral nutrition under low soil fertility (Lambers et al. 2008), and grazing has adverse effects on these associations through photosynthate limitation (Bardgett and Wardle 2003).

In contrast to ANPP, BNPP was not influenced by soil resource availability, grazing intensity, or plant functional diversity, and it varied independently of ANPP. Measuring BNPP is notoriously difficult and the root ingrowth method used in our study, while better than sequential coring, can underestimate BNPP due to fine-root turnover (Milchunas 2009). Coupled with limited subsampling (i.e., two rounds of four ingrowth cores per plot), we cannot exclude the possibility that the lack of significant relationships may be due to high measurement error. Nevertheless, the unresponsiveness of BNPP to changes in soil resource availability may have arisen because plants generally allocate a greater proportion of their resources to root growth under lower soil resource availability, in order to match aboveground demands (Lambers et al. 2008). Moreover, the absence of impacts of grazing is consistent with previous findings (McNaughton et al. 1998).

Litter decomposition was strongly related to ANPP, but was only indirectly influenced by changes in specific

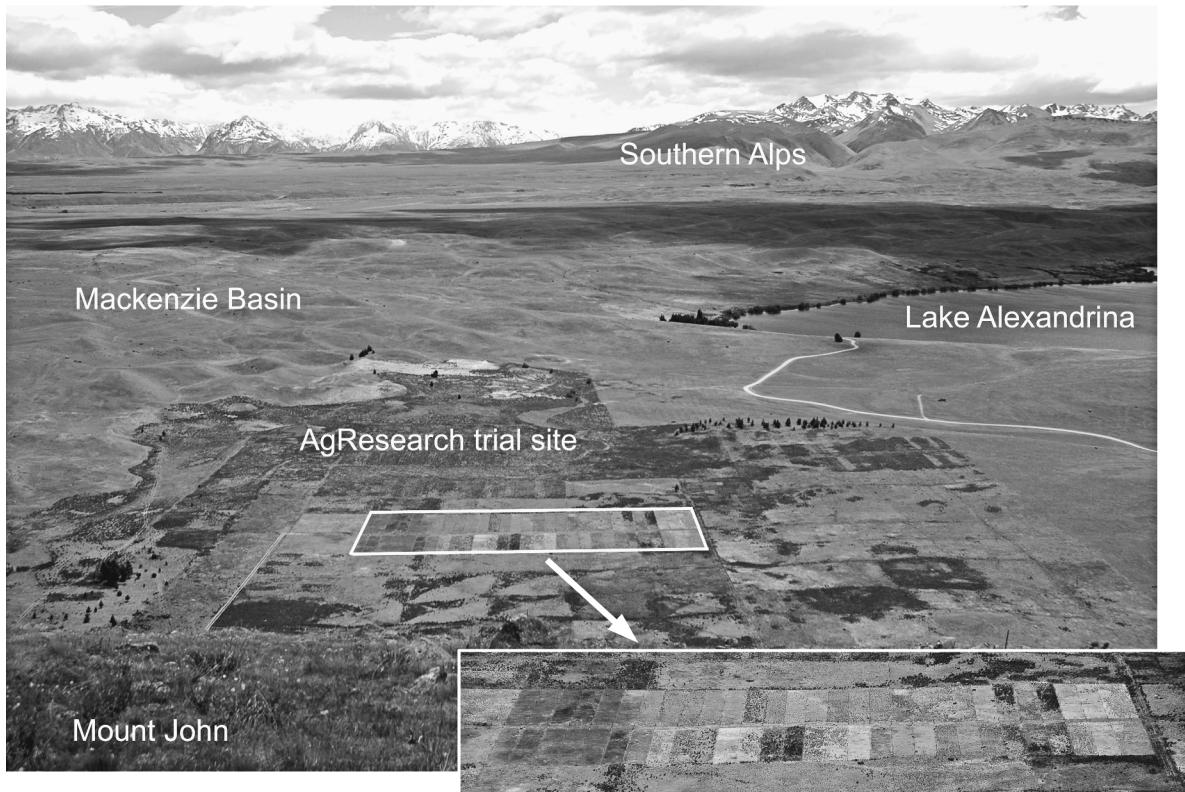


PLATE 1. Aerial view of the study area and AgResearch trial site at Mount John, located in the Mackenzie Basin of the South Island of New Zealand. The white rectangle shows the location of the experiment used in the present study (inset: close-up view of the  $8 \times 50$  m experimental plots). Photo credits: E. Laliberté.

leaf area, despite several studies showing that plants with higher specific leaf area produce litter that decomposes more rapidly (e.g., Garnier et al. 2004, Queded et al. 2007, Quétier et al. 2007, Cornwell et al. 2008). Our results thus point toward greater rates of primary production promoting the activity of decomposers. However, we also found litter decomposition rate within plots (i.e., the data used in the structural equation model; Fig. 1) to be very strongly correlated ( $r = 0.964$ ,  $P < 0.0001$ ) with litter decomposition rate under standard soil conditions (Appendix F). This means that the strong effect of ANPP on litter decomposition in our model must hide effects of litter traits that covary with ANPP, but are not adequately captured by specific leaf area. Such litter traits may include concentration of labile carbon compounds, e.g., nonstructural carbohydrates or phenolics (Aerts et al. 2003, Meier and Bowman 2008, Hättenschwiler et al. 2011).

Both ANPP and BNPP were associated with increases in soil carbon in the 0–20 cm depth layer, presumably through greater plant-derived inputs to the soil (De Deyn et al. 2008). Grazing intensity was also found to have a strong, direct, positive effect on soil carbon. This result is counterintuitive because higher grazing intensity often increases soil respiration, which is generally linked

to soil carbon loss (De Deyn et al. 2008, Klumpp et al. 2009). Nevertheless, this may occur if defoliation-induced increases in root exudation (Hamilton et al. 2008) not only stimulated microbial biomass but also promoted the formation of soil microaggregates (Jones and Alison 2004, De Deyn et al. 2008). The positive relationship between specific leaf area and surface soil carbon, although difficult to interpret, may have arisen because rhizodeposition and fine-root turnover tend to be greater for faster-growing species (De Deyn et al. 2008, Lambers et al. 2008), which can in turn enhance soil carbon storage (De Deyn et al. 2008). It has been suggested that a trait-based approach could enhance our understanding of the processes controlling soil carbon storage, but that because measuring root traits can be challenging, identifying easily measurable aboveground traits that capture belowground processes is an important area for future research (De Deyn et al. 2008). Our results suggest that specific leaf area may act as an easily measured proxy for root traits that influence soil carbon storage; still, much more work is needed to confirm this preliminary result.

We also found that soil resource availability had a dominant, nonlinear effect on the amount of carbon stored in the soil surface (0–20 cm depth). Soil carbon increased progressively with fertilization rate, but then

dropped sharply at the highest resource availability level, which was also irrigated and sustained by far the greatest ANPP (Appendix G). A limited rooting-depth distribution can restrict opportunities for buildup of soil carbon (De Deyn et al. 2008), and indeed we found a significantly greater proportion of BNPP restricted to the 0–10 cm soil layer at the highest soil resource availability (Appendix H). Moreover, because our study area frequently experiences summer moisture deficits, which can effectively halt soil respiration, irrigation may have promoted soil carbon loss by increasing soil respiration on an annual scale. Greater leaching losses of carbon in the surface soil layer under irrigation also cannot be excluded.

Soil carbon residing at depth provides a more stable carbon pool (Jones and Alison 2004). Because very few roots were found beyond 40 cm, the amount of soil carbon stored at greater depth (60–80 cm) is expected to originate mainly through leaching from upper soil layers (Jones and Alison 2004). Accordingly, it was primarily driven by surface soil carbon. However, soil resource availability also strongly, and linearly, decreased deeper carbon stores. This may occur if more labile organic matter is returned to the soil under greater resource availability (De Deyn et al. 2008), restricting opportunities for the buildup of recalcitrant organic matter in deeper soil layers. Together, our results for soil carbon at the surface (0–20 cm) and at greater depth (60–80 cm) illustrate that increased plant production through high fertilization and irrigation does not necessarily translate, in the long term, into greater soil carbon sequestration in grasslands (Jones and Alison 2004).

In conclusion, our results from this long-term experiment suggest that by altering the bottom-up (resources) and top-down (herbivory) forces that structure ecosystems, land-use intensification has cascading effects on ecosystem functioning, both through direct effects of changes to resource availability (Burke et al. 1997, Díaz et al. 2007, Lambers et al. 2008) and grazing, and through the indirect effects of these environmental forces on plant traits (Chapin et al. 2000, Hooper et al. 2005, Díaz et al. 2007) and functional diversity (Hooper et al. 2005, Díaz et al. 2007). The pivotal role of ANPP in governing other functions in our model supports the idea that it is a key integrator of ecosystem functioning (McNaughton et al. 1989). Importantly, soil resource availability not only had the strongest influence on ecosystem functioning, but it also modulated the effects of both plant functional diversity (Fridley 2002, Wardle and Zackrisson 2005) and grazing intensity (Bardgett and Wardle 2003).

Our study may help to reconcile the disparity between the productivity–diversity patterns found in natural systems (Huston 1994) and the diversity–productivity relationships found in biodiversity–ecosystem function experiments (Hooper et al. 2005). On one hand, our finding that functional diversity was negatively related to ANPP under the lower fertility levels invites the

interpretation that, under such conditions, higher diversity actually results from lower production and associated slower rates of competitive exclusion (Huston 1994). On the other hand, our results suggest that functional diversity may be important for ecosystem functioning, even against the background of other abiotic and biotic drivers (Srivastava and Vellend 2005), provided that soil resource availability is high (Fridley 2002). This supports the idea that functionally diverse species mixtures can improve the efficiency of production systems, such as intensive pastures (Sanderson et al. 2004) or biofuel crops on abandoned agricultural land (Fargione et al. 2008).

Grasslands are expected to undergo rapid intensification of grazing pressure and fertilizer use in the coming decades to meet the growing global demand for livestock products (Bouwman et al. 2005). Our results show that increasing grazing pressure in marginal grasslands can negatively influence ecosystem functioning, potentially compromising long-term production potential. Conversely, large concomitant increases in agricultural inputs (especially irrigation) and grazing intensity have clear synergistic positive effects on production, yet restrict opportunities for soil carbon sequestration. Our study illustrates how understanding the direct, indirect, and interactive effects of land-use changes on communities and ecosystems can help us to better assess and balance such inherent trade-offs among multiple ecosystem functions (Foley et al. 2005).

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#### LITERATURE CITED

- Aerts, R. 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant–soil feedbacks. *Journal of Experimental Botany* 50:29–37.
- Aerts, R., H. De Caluwe, and B. Beltman. 2003. Plant community mediated vs. nutritional controls on litter decomposition rates in grasslands. *Ecology* 84:3198–3208.
- Augustine, D. J., and S. J. McNaughton. 2007. Interactive effects of ungulate herbivores, soil fertility, and variable rainfall on ecosystem processes in a semi-arid savanna. *Ecosystems* 9:1242–1256.
- Bagchi, S., and M. E. Ritchie. 2010. Introduced grazers can restrict potential soil carbon sequestration through impacts on plant community composition. *Ecology Letters* 13:959–968.

- Balvanera, P., A. B. Pfisterer, N. Buchmann, J.-S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters* 9:1146–1156.
- Bardgett, R. D., and D. A. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* 84:2258–2268.
- Bouwman, A. F., K. W. Van der Hoek, B. Eickhout, and I. Soenar. 2005. Exploring changes in world ruminant production systems. *Agricultural Systems* 84:121–153.
- Burke, I. C., W. K. Lauenroth, and W. J. Parton. 1997. Regional and temporal variation in net primary production and nitrogen mineralization in grasslands. *Ecology* 78:1330–1340.
- Chapin, F. S. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11:233–260.
- Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavelle, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. *Nature* 405:234–242.
- Collins, S. L., A. K. Knapp, J. M. Briggs, J. M. Blair, and E. M. Steinauer. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science* 280:745–747.
- Cornelissen, J. H. C., S. Lavelle, E. Garnier, S. Diaz, N. Buchmann, D. E. Gurvich, P. B. Reich, H. ter Steege, H. D. Morgan, M. G. A. van der Heijden, J. G. Pausas, and H. Poorter. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* 51:335–380.
- Cornwell, W. K., et al. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* 11:1065–1071.
- Craine, J. M., and W. G. Lee. 2003. Covariation in leaf and root traits for native and non-native grasses along an altitudinal gradient in New Zealand. *Oecologia* 134:471–478.
- De Deyn, G. B., J. H. C. Cornelissen, and R. D. Bardgett. 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters* 11:516–531.
- Díaz, S., S. Lavelle, F. de Bello, F. Quétier, K. Grigulis, and T. M. Robson. 2007. Incorporating plant functional diversity effects in ecosystem service assessments. *Proceedings of the National Academy of Sciences USA* 104:20684–20689.
- Fargione, J., J. Hill, D. Tilman, S. Polasky, and P. Hawthorne. 2008. Land clearing and the biofuel carbon debt. *Science* 319:1235–1238.
- Foley, J. A., et al. 2005. Global consequences of land use. *Science* 309:570–574.
- Fridley, J. D. 2002. Resource availability dominates and alters the relationship between species diversity and ecosystem productivity in experimental plant communities. *Oecologia* 132:271–277.
- Fridley, J. D. 2003. Diversity effects on production in different light and fertility environments: an experiment with communities of annual plants. *Journal of Ecology* 91:396–406.
- Garnier, E., J. Cortez, G. Billès, M.-L. Navas, C. Roumet, M. Debussche, G. Laurent, A. Blanchard, D. Aubry, A. Bellmann, C. Neill, and J.-P. Toussaint. 2004. Plant functional markers capture ecosystem properties during secondary succession. *Ecology* 85:2630–2637.
- Garnier, E., et al. 2007. Assessing the effects of land-use change on plant traits, communities and ecosystem functioning in grasslands: a standardized methodology and lessons from an application to 11 European sites. *Annals of Botany* 99:967–985.
- Garnier, E., B. Shipley, C. Roumet, and G. Laurent. 2001. A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Functional Ecology* 15:688–695.
- Grace, J. B., et al. 2007. Does species diversity limit productivity in natural grassland communities? *Ecology Letters* 10:680–689.
- Grime, J. P. 1973. Competitive exclusion in herbaceous vegetation. *Nature* 242:344–347.
- Hamilton, E. W., D. A. Frank, P. M. Hinchey, and T. R. Murray. 2008. Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. *Soil Biology and Biochemistry* 40:2865–2873.
- Hättenschwiler, S., S. Coq, S. Barantal, and I. T. Handa. 2011. Leaf traits and decomposition in tropical rainforests: revisiting some commonly held views and towards a new hypothesis. *New Phytologist* 189:950–965.
- Hewitt, A. E. 1998. *New Zealand soil classification*. Manaaki Whenua Press, Lincoln, New Zealand.
- Hillebrand, H., and B. Matthiessen. 2009. Biodiversity in a complex world: consolidation and progress in functional biodiversity research. *Ecology Letters* 12:1405–1419.
- Hooper, D. U., et al. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75:3–35.
- Huston, M. A. 1994. *Biological diversity*. Cambridge University Press, Cambridge, UK.
- Jones, M. B., and D. Alison. 2004. Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO<sub>2</sub>. *New Phytologist* 164:423–439.
- Klump, K., S. Fontaine, E. Attard, X. Le Roux, G. Gleixner, and J.-F. Soussana. 2009. Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community. *Journal of Ecology* 97:876–885.
- Laliberté, E., and P. Legendre. 2010. A distance-based framework for measuring functional diversity from multiple traits. *Ecology* 91:299–305.
- Laliberté, E., D. A. Norton, J. M. Tylianakis, and D. Scott. 2010. Comparison of two sampling methods for quantifying changes in vegetation community structure under rangeland development. *Rangeland Ecology and Management* 63:537–545.
- Lambers, H., F. S. Chapin, and T. L. Pons. 2008. *Plant physiological ecology*. Springer, New York, New York, USA.
- Lauenroth, W. K. 2000. Methods of estimating belowground net primary production. Pages 58–71 in O. E. Sala, R. B. Jackson, H. A. Mooney, and R. W. Howarth, editors. *Methods in ecosystem science*. Springer, New York, New York, USA.
- Lavelle, S., and E. Garnier. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology* 16:545–556.
- McGlone, M. S. 2001. The origin of native grasslands of southeastern South Island in relation to pre-human woody ecosystems. *New Zealand Journal of Ecology* 25:1–15.
- McNaughton, S. J. 1983. Compensatory plant growth as a response to herbivory. *Oikos* 40:329–336.
- McNaughton, S. J., F. F. Banyikwa, and M. M. McNaughton. 1997. Promotion of the cycling of diet-enhancing nutrients by African grazers. *Science* 278:1798–1800.
- McNaughton, S. J., F. F. Banyikwa, and M. M. McNaughton. 1998. Root biomass and productivity in a grazing ecosystem: the Serengeti. *Ecology* 79:587–592.
- McNaughton, S. J., D. G. Milchunas, and D. A. Frank. 1996. How can net primary productivity be measured in grazing ecosystems? *Ecology* 77:974–977.
- McNaughton, S. J., M. Oesterheld, D. A. Frank, and K. J. Williams. 1989. Ecosystem-level patterns of primary productivity and herbivory in terrestrial habitats. *Nature* 341:142–144.
- Meier, C. L., and W. D. Bowman. 2008. Links between plant litter chemistry, species diversity, and below-ground ecosystem function. *Proceedings of the National Academy of Sciences USA* 105:19780–19785.

- Milchunas, D. G. 2009. Estimating root production: comparison of 11 methods in shortgrass steppe and review of biases. *Ecosystems* 12:1381–1402.
- Olf, H., and M. E. Ritchie. 1998. Effects of herbivores on grassland plant diversity. *Trends in Ecology and Evolution* 13:261–265.
- Olson, J. S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. *Ecology* 44:322–331.
- Pimm, S. L., and P. Raven. 2000. Extinction by numbers. *Nature* 403:843–845.
- Quested, H., O. Eriksson, C. Fortunel, and É. Garnier. 2007. Plant traits relate to whole-community litter quality and decomposition following land use change. *Functional Ecology* 21:1016–1026.
- Quétier, F., A. Thébault, and S. Lavorel. 2007. Plant traits in a state and transition framework as markers of ecosystem response to land-use change. *Ecological Monographs* 77:33–52.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Reich, P. B., M. B. Walters, and D. S. Ellsworth. 1992. Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecological Monographs* 62:365.
- Sala, O. E., et al. 2000. Global biodiversity scenarios for the year 2100. *Science* 287:1770–1774.
- Sanderson, M. A., R. H. Skinner, D. J. Barker, G. R. Edwards, B. F. Tracy, and D. A. Wedin. 2004. Plant species diversity and management of temperate forage and grazing land ecosystems. *Crop Science* 44:1132–1144.
- Scott, D. 1999. Sustainability of New Zealand high-country pastures under contrasting development inputs. 1. Site and shoot nutrients. *New Zealand Journal of Agricultural Research* 42:365–383.
- Scott, D. 2001. Sustainability of New Zealand high-country pastures under contrasting development inputs. 7. Environmental gradients, plant species selection, and diversity. *New Zealand Journal of Agricultural Research* 44:59–90.
- Shipley, B. 2009. Confirmatory path analysis in a generalized multilevel context. *Ecology* 90:363–368.
- Srivastava, D. S., and M. Vellend. 2005. Biodiversity–ecosystem function research: is it relevant to conservation? *Annual Review of Ecology and Systematics* 36:267–294.
- Wardle, D. A., and O. Zackrisson. 2005. Effects of species and functional group loss on island ecosystem properties. *Nature* 435:806–810.
- Wright, I. J., et al. 2004. The worldwide leaf economics spectrum. *Nature* 428:821–827.

#### SUPPLEMENTAL MATERIAL

##### Appendix A

Full multivariate model and its justification (*Ecological Archives* E093-014-A1).

##### Appendix B

Details on the estimation of litter decomposition rates (*Ecological Archives* E093-014-A2).

##### Appendix C

Details on generalized multilevel path models (*Ecological Archives* E093-014-A3).

##### Appendix D

Figure showing the relationship between specific leaf area and soil resource availability (*Ecological Archives* E093-014-A4).

##### Appendix E

Figure showing the relationship between surface soil carbon and soil resource availability (*Ecological Archives* E093-014-A5).

##### Appendix F

Figure showing the relationship between litter decomposition rate within plots and litter decomposition under standard soil conditions (*Ecological Archives* E093-014-A6).

##### Appendix G

Figure showing the relationship between ANPP and soil resource availability (*Ecological Archives* E093-014-A7).

##### Appendix H

Figure showing that resource availability alters the depth distribution of belowground net primary production, BNPP (*Ecological Archives* E093-014-A8).