

DISSERTATION

PLANT-SOIL-WATER RELATIONS IN THREE CONTRASTING ENVIRONMENTS

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ABSTRACT

PLANT-SOIL-WATER RELATIONS IN THREE CONTRASTING ENVIRONMENTS

The relationships among soil nutrients, water availability, and vegetation can be studied over spatial and temporal scales that vary by orders of magnitude. Each provides a particular set of insights into tightly linked ecosystem dynamics. I examined the effects of rainfall on soil CO₂-C efflux in a semi-arid woodland, the ramifications of ungulate herbivory for soil nutrients and microbial processes in a montane riparian zone, and the consequences of manipulating soil nutrient levels and vegetation density for carbon isotope discrimination in a wet tropical forest.

Soil CO₂-C efflux responded more strongly to a natural change in the timing and magnitude of precipitation that occurred during the study than to irrigation or drought treatments, indicating the importance of the timing of rainfall. Soil CO₂-C efflux declined with artificial drought under trees and in open locations; the patterns and rates of the response differed between the two locations. Maximum efflux rates under trees occurred with supplemental irrigation; maximum rates in open locations occurred with ambient rainfall and artificial warming.

Soil nutrient concentrations and quantities were significantly lower in grazed plots compared to paired plots that had been protected from grazing for 15 years. Low levels of nutrients were associated with higher bulk density and lower soil moisture in the grazed areas.

Carbon isotopes from wood cellulose of trees in an experimental wet tropical forest were invariant across treatments, contrasting with significant treatment-related differences in gross primary production. I consider several reasons why discrimination might not co-vary with gross primary production, including decreased isotopic response when conductance is not limiting and the muting of signals in wood as compared to foliage.

Each experiment reflected at least three years of treatment effects and included data ranging from the temporally and spatially integrated values of carbon isotopes from wood-tree rings and annual soil CO₂-C efflux to the point processes captured by measurements of microbial biomass and fatty acids. Together, they demonstrate various sensitivities to environmental modifications that should increase our understanding of plant-soil-water relationships.

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CHAPTER 1: INTRODUCTION

The study of energy, water, and nutrient fluxes within and between the biotic and abiotic environments defines ecosystem ecology (Chapin *et al.*, 2002). Experimental manipulation of these fluxes can improve our understanding of the processes that control the distribution and abundance of organisms, the fundamental objective of ecology (Krebs, 1978). I conducted three studies in which water or nutrient processes were experimentally altered. Two of the studies addressed nutrient and water fluxes within the context of community structure and function; the third study is a melding of ecosystem and physiological ecology that examines the relationship between water and nutrient availability and plant function. Together, the three research projects demonstrate the importance of water as a mediator of nutrient availability, whether the nutrients are atmospheric carbon dioxide (CO₂) or the carbon contained in litterfall.

Linkages among energy, water, and nutrient fluxes exist in a variety of forms. At the plant physiological level, the balance between soil water availability and atmospheric water availability (vapor pressure deficit) maintains the stomatal opening necessary for CO₂ uptake (Taiz *et al.*, 2006). Water availability is thus a precursor for nutrient uptake. In soils, by contrast, water is the medium for nutrient availability, transporting dissolved nutrients to soil organisms or fine roots. Water is essential for maintaining physiological function for organisms in the soil-plant-atmosphere continuum; insufficient water can cause hydraulic failure in plants (Tyree *et al.*, 2002) or induce dormancy in soil

organisms (Sylvia, 2005). Excessive amounts of water can create anoxic conditions favorable neither to plant roots nor to aerobic soil organisms.

The connections among water availability, nutrient fluxes, and organismal function necessarily engender close associations among changes in moisture levels and in ecosystem structure and function (Aber *et al.*, 2001). In Chapter 1, I examine the effects of experimental drought on soil respiration in a semi-arid woodland. The death of many trees in the experimental drought treatments increased litterfall, increasing food availability for soil organisms. Contrary to expectation but consistent with the changes in ecological structure, rates of soil CO₂-C efflux did not differ significantly between the drought and the ambient treatments; tree mortality in the drought treatments was associated with a decrease in plant-available water but with higher water availability for heterotrophic soil organisms, as well as an influx of nutrients from litterfall.

Water and nutrient fluxes in the soil-plant interface can be modified indirectly by grazing animals (*e.g.*, Pastor *et al.* 1993, Knapp *et al.* 1999) as well as by the direct effects of climate. Native ungulates can translocate nutrients between grazing and bedding areas (Schoenecker *et al.* 2004), change community composition through preferential grazing (*e.g.*, Pastor *et al.* 1993), or alter the physical environment by their presence, as by compacting the soil (Drewry *et al.* 1999). In Chapter 2, I examine the relationship between the presence or absence of grazing and soil nutrients, physical soil properties, and soil microbial biomass and composition. The higher bulk density observed in the grazed areas is associated with lower soil moisture and lower nutrient levels, likely as a result of decreases in nutrient transport within the soil.

The two studies briefly described above illustrate circumstances in which linked changes in water and nutrient fluxes are altering ecosystem structure and function, with unknown potential for recovery to the initial state. Extreme sensitivity to increases or decreases in water availability is most often seen where water stress limits both physiological function and nutrient acquisition (Aber *et al.* 2001). When water is plentiful, the coupling between function and nutrient acquisition might be weaker.

In the final chapter, I present data from an experimental forest of *Eucalyptus saligna* in Hawaii, where annual rainfall exceeds 4000 mm yr⁻¹. Forest trees were subjected to treatments including three levels of fertilization and two levels of density. Fertilization caused increased gross primary production and lead to earlier canopy closure (Ryan *et al.*, 2004). When soil or atmospheric water is limited, increases in photosynthetic activity associated with greater nutrient availability can theoretically decrease the CO₂ concentration in the leaf intercellular spaces and the extent of discrimination against ¹³C (MacFarlane *et al.*, 2004). In a water-limited environment, increased nutrient uptake might therefore decrease carbon isotope discrimination (Δ). Wood cellulose Δ did not change, however, as gross primary production increased. This could be because the combination of high soil water availability and high atmospheric water availability precluded the stomatal closure typical of drier environments, maintaining CO₂ uptake. This study could therefore indicate that high water availability limits physiological sensitivity to increased nutrient fluxes. I additionally consider whether or not it is appropriate to use wood cellulose Δ as an indicator of physiological function.

Among the three case studies described above, one – the grazing study - showed biologically and statistically significant effects related to the experimental manipulations. The greater soil bulk density associated with grazing appears to have initiated a sequence of events for which there were no compensatory processes; the result was net loss of soil nutrients. In the other two situations, changes in water or nutrient fluxes either gave rise to structural changes that maintained the flux being measured – soil CO₂-C or stomatal CO₂-C – or had no measureable effect.

The frequency of natural and anthropogenic modification of ecosystem water and nutrient fluxes and pools is likely to increase during the coming decades (Chapin III *et al.* 2009). The functional and structural responses observed in two of the three case studies that constitute this dissertation exemplify interactions between soil water and nutrients and vegetation that remain poorly understood. Such interactions can equally herald major changes in ecosystem services, structure, and function (Chapin III *et al.* 2009) or result in no change when consequences were anticipated. Since two of the projects are ongoing, additional data collection in the coming years may further clarify the current results and continue to contribute to our understanding of the water, energy, and nutrient fluxes that unite physiological, community, and ecosystem ecology.

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CHAPTER 2

Soil respiration responses to altered precipitation in a semi-arid woodland

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Abstract

Semi-arid regions have a history of variable rainfall and vegetative structure that is expected to continue or accelerate, with likely ramifications for the amount of carbon that diffuses from soil as carbon dioxide (soil CO₂-C efflux) and soil carbon (C) storage. We manipulated moisture availability in a semi-arid woodland in the southwestern United States for three years and studied the effects on soil CO₂-C efflux under tree canopies and in open locations. We decreased precipitation by 50% compared to ambient and increased precipitation by 20% in 40m x 40 m replicated plots. An interannual decline in ambient rainfall of approximately the same magnitude as the irrigation treatment occurred during the study. The natural 20% decline in precipitation from 2007 to 2008 was contemporaneous with a large decrease in soil CO₂-C efflux rates in all experimental treatments, both under tree canopies and in the open. Annual CO₂-C efflux under *Pinus edulis* in the ambient treatment decreased from 575 (CV 0.14) g C m⁻² yr⁻¹ to 398 (CV 0.26) g C m⁻² yr⁻¹ from 2007 to 2009; the decrease in open locations was from 410 (CV 0.46) g C m⁻² yr⁻¹ to 208 (CV 0.06) g C m⁻² yr⁻¹. Experimental precipitation levels in the irrigation plots in 2008 and 2009 were similar to ambient rainfall in 2007 but soil CO₂-C efflux rates did not recover to 2007 levels (488 (CV 0.41) g C m⁻² yr⁻¹ in open locations in 2007 and 241 (CV 0.13) g C m⁻² yr⁻¹ in 2009). The spatial pattern created by the presence or absence of a tree canopy accounted for more variation in mean annual soil CO₂-C efflux rates than did the treatments (431 (CV 0.09) g C m⁻² yr⁻¹ under trees in irrigated treatments in 2009 and 241 (CV 0.13) g C m⁻² yr⁻¹ in the open locations). The experimental infrastructure warmed the soil in the drought and control treatments. The death of trees in the drought plots was followed by increasing soil moisture under the

newly-opened tree canopies. We discuss the relationships among soil moisture, soil respiration, and ecological structure in the context of changing rainfall regimes.

Introduction

The rate of carbon dioxide (CO₂) efflux from the soil to the atmosphere is affected by soil moisture, soil temperature, and aboveground vegetation (Wildung *et al.*, 1975; Anderson, 1991; Raich *et al.*, 2000,). In the semi-arid regions of the world, these driving factors have exhibited large historical variability that could increase with changing atmospheric CO₂ levels (Anderson, 1991; Stahle, 2000; Batisani *et al.*, 2010). Soil CO₂ efflux is an integrated gauge of soil biological and physical processes and can indicate changes in soil carbon storage rates when combined with other data. Our understanding of how the carbon cycle responds to change is largely premised on balance and constancy and does not reflect today's non-steady state conditions (Chapin III *et al.*, 2009).

Changes in land cover are often particularly rapid in semi-arid woodlands, savannas, or parklands, where the balance of trees, grasses, and shrubs can vary at sub-decadal time scales (Allen *et al.*, 1998, Bucini *et al.*, 2007). We cannot predict the permanence of such rapid transitions but we can increase our understanding of the consequences for ecosystem function by quantifying the component responses. Experimental manipulations with water are increasingly used to study the effects of changing rainfall on soil CO₂ efflux and other ecosystem processes (*e.g.*, Gilgen *et al.*, 2009; Heisler-White *et al.*, 2009; Misson *et al.*, 2010; Unger *et al.*, 2010). Research that compares the response by vegetation type (*e.g.*, McCulley *et al.*, 2007) is less common but might provide greater predictive opportunities.

We manipulated the amount of precipitation available to overstory and understory plants in a semi-arid woodland in the southwestern United States for three years and monitored the changes in soil CO₂ efflux rates by treatment, year, and vegetation type.

We expected soil CO₂ efflux to decline with soil moisture in the drought treatments because heterotrophic and autotrophic respiration would become water-limited. We also expected the irrigation treatment to stimulate net primary production and to reduce the water-limitation to heterotrophic respiration, resulting in increased rates of soil CO₂ efflux.

Materials and Methods

The research site is a semi-arid woodland in central New Mexico, USA, on the eastern boundary of the Sevilleta Long Term Ecological Research site, Sevilleta National Wildlife Refuge (34°N, 107°W); the elevation is 1800 m. Precipitation is bimodal with snow and rain in the winter and brief intense rains in the summer; mean annual precipitation is approximately 300 mm. Spring and fall are typically dry with strong winds during March and April. Mean daily temperature ranges from 2°C in the winter to 25°C during the summer. Soils are shallow gravelly silt loams and sandy loams with a hardpan within 40-100 cm of the surface. Soils average 1.6% carbon with a C:N ratio of 18.

Overstory vegetation was primarily pinyon pine (*Pinus edulis*) and one-seed juniper (*Juniperus monosperma*). Mean woody basal area per plot within a treatment ranged from 6.8 m² to 11.3 m². Juniper accounted for at least 90% of the woody basal area in each treatment. Mature pinyon trees were over 200 years old; juniper were large and of indeterminate age (P.M. Brown, *pers. comm.*). The understory was predominately perennial C₄ grasses, including black grama (*Bouteloua eriopoda*), hairy grama (*Bouteloua hirsuta*), and sideoats grama (*Bouteloua curtipendula*). Shrubs included

creeping barberry (*Mahonia repens*) and shrub oak (*Quercus turbinella*). Prickly pear and cholla (*Opuntia* and *Cylindropuntia* spp.) were also common. Overall cover at the site was estimated as 7.8% pinyon canopy, 31.3% juniper canopy, and 60.9% grassy or open areas without tree canopy (White 2008).

Experimental design and measurements

Twelve 40m x 40m plots were installed at the site. Treatments were ambient, irrigated, drought, and infrastructure control, with three replicates of each. One replicate of each treatment was on a north facing slope, one on flat terrain, and one on a south facing slope. Irrigated plots had 16 6-m tall sprinkler poles and were to receive precipitation additions that would increase annual precipitation by 50% compared to the 30 year mean. Drought plots had a rain gutter design modified from Yahdjian and Sala (2002). The gutters were approximately one meter above the ground and formed a series of parallel troughs intended to capture 50% of ambient precipitation; the remaining precipitation fell to the ground between the troughs. The Makrolon SL polycarbonate (Sheffield Plastics, Inc) troughs had an ultra-violet resistant coating and transmitted 86% of total light. Infrastructure control plots were similar to the drought plots but the troughs were inverted. The purpose of the control was to document effects caused by the troughs rather than by the treatments. Irrigation and drought infrastructure were completed during summer, 2007. The first irrigation treatment was in June, 2008.

Meteorological data were collected 2m above the ground surface at a weather station located on a hilltop between two treatment plots. Data collected included relative humidity and air temperature (CS-215-L, Campbell Scientific), net radiation (CS-300-L,

Campbell Scientific), photosynthetically active radiation (LI1905B-L, LI-COR), wind speed (CS05305-L, Campbell Scientific), and precipitation.

Soil CO₂ efflux sampling

Spatial variability in soil carbon dioxide efflux was captured during planned monthly measurements from late 2006 through 2009; actual sampling frequency was somewhat less due to factors such as weather conditions and equipment failure (Table S1, Supporting information). Nine polyvinyl chloride collars were installed per plot. Three collars were installed under each of pinyon canopy and juniper canopy, and three in open areas without canopy cover. Collars had an outer diameter of 254 mm and were inserted between 3 cm and 5 cm into the ground. Collars remained in place from 2006 through 2009 and were reseated when disturbed. Fourteen of the 108 collars had to be moved to new locations during the study due to disturbance of the surrounding soil. Measurements were made by sealing the collars with an opaque vented domed cap attached to a LI-6200 infrared gas analyzer (Li-Cor, Lincoln, Nebraska, USA). In 2009, an LI-820 (Li-Cor, Lincoln, Nebraska, USA) was used when the LI-6200 was being serviced. In both cases, the system was closed; measurements commenced when efflux rates were stable and at ambient CO₂. All collars in each plot were sampled from 2006 through mid 2007 and from late 2008 through 2009. Six collars (two under juniper, two under pinyon, and two in the open) were sampled from mid 2007 through mid 2008. From 2006 through mid 2008, soil efflux measurements were made over a 4 ppm change in CO₂ concentration, or 3 ppm during the winter. From late 2008 through 2009, soil efflux measurements were taken for 30 seconds, examined for non-linearity, and retaken if the r^2 was less than 0.96.

Measurement periods based on change in ppm or based on time are both possible on the LI-6200, depending on programming input by the user.

Soil volumetric water content and soil temperature were measured during each soil efflux measurement until late 2008. Handheld measurements were made with a time domain reflectometer (Hydrosense, Campbell Scientific, Logan, Utah, USA) and a thermocouple temperature sensor (HH509, Omega Engineering, Stamford, Connecticut, USA). The manual soil moisture measurements from 2007 and 2008 were subsequently discarded due to technical errors during measurement. Sensors for automated collection of soil temperature and moisture data became operational during 2007. Automated soil moisture measurements were made with ECH₂O soil moisture sensors (Model EC-20, Decagon Devices, Pullman, Washington, USA) installed 5 cm below and parallel to the soil surface. Soil temperature was measured by thermocouples buried 5 cm below the soil surface near each collar. Air temperatures were recorded by shielded thermocouples approximately 20 cm above the soil surface. All automated measurements were transmitted via wireless network to a server maintained by the University of New Mexico. Measurement frequency varied during the three year period but was at least every 30 minutes.

Monthly soil CO₂ efflux sampling was supplemented with high frequency measurements from a pneumatically-operated closed chamber system (autochamber) based on the design of Wayson *et al.* (2006) (Design Metal Manufacturing, Fort Collins, Colorado USA). Chambers were clear Lexan cylinders with a diameter of 254 mm and extended 200 mm above the soil surface. Two chambers were placed in one plot of each treatment type in late 2006, for a total of 8 chambers. One chamber in each plot was

installed under a pinyon canopy and one was placed in an open area without canopy cover. The chambers in the infrastructure control plots were discontinued in late 2008. A chamber was added under juniper canopy in the ambient, irrigated, and drought plots in September, 2009. The autochambers were controlled by a CR-10X (Campbell Scientific, Logan, Utah, USA) programmed to sample the chambers sequentially at regular intervals; each chamber was sampled between 12 and 20 times daily, depending on the number of chambers in place and the sampling time per chamber. The changing carbon dioxide concentration in the closed chamber was measured by a LI-800 infrared gas analyzer (LI-COR, Lincoln, Nebraska, USA). Autochambers were closed for 10 minutes, with flux rates determined on the last 3 minutes of data. Soil temperature data were transmitted from a thermocouple wire buried near each chamber. Barometric pressure, air temperature, and volumetric soil moisture were recorded at a central location near the CR10-X and the LI-800. Data were transmitted wirelessly every 30 minutes to a server maintained by the University of New Mexico. The autochamber system was powered by a solar array with battery storage. The system was operational during most of 2007 and sporadically in 2008. In late 2008, the system was removed for rebuilding, with full function restored in August, 2009.

Soil carbon inputs

We collected litterfall in 32.5 cm x 41.5 cm trays during each year of the study. Fifteen trays were installed in one plot of each treatment and 8 trays were installed in the other two plots of each treatment. Trays were installed under trees and in the open spaces. The trays that were not under canopy cover were removed in 2009; litter collected in the open spaces was not included in the calculations of C inputs from litter. Litterfall from trees

was not separated by species. Litterfall was collected twice in 2007 and once in 2009. Litter was oven-dried to constant mass and weighed. We used a conversion factor of 50% (Klopatek *et al.*, 1995, Gehring *et al.*, 1997) to convert dry litter weight to grams of C. We calculated a net C flux for the treed portion of the woodland study site by subtracting annual soil C efflux under tree canopies from annual litterfall C in the wooded portions of the plots.

We estimated grass production in 2009 by clipping 3 randomly placed 1 m² subplots in each treatment plot at the end of the growing season. Grasses were dried to constant mass and weighed. Production was not adjusted for losses to herbivory because grazing exclosures had not been in place long enough to provide a reliable estimate.

Estimates of annual soil CO₂ efflux

We estimated total soil C efflux by combining data from the automated chamber measurements and the monthly collar measurements, using the approach of Litton *et al.* (2003). We assessed the sensitivity of soil C efflux to soil temperature by inspecting data from each autochamber and identifying periods of at least 6 days when soil moisture was not changing. We then fit data from the individual time periods to the exponential model:

$$F_s = \alpha * e^{\beta * T} \quad (1)$$

where T is the soil temperature at the time of each flux measurement and α and β are fitted constants (Lloyd *et al.*, 1994). By linearizing the model and using a logarithmic fit, we obtained r^2 values for assessing model quality. We found between one and five time periods for each autochamber that were suitable for determining β and Q_{10} . If more than one time period was available, we averaged β values to get one coefficient for each

treatment and cover type combination; we examined the data for trends with time or treatment-related changes in β before averaging. We used the average β for each treatment and cover type combination to estimate Q_{10} , the rate of increase in soil C efflux for each 10°C increase in soil temperature:

$$Q_{10} = e^{\beta * 10} \quad (2)$$

We adjusted the monthly soil efflux data to a common temperature to eliminate the bias that results from taking measurements at different times of day and estimated soil efflux on each day between collar measurements with linear interpolation. The standardized interpolated measurements were finally adjusted to the actual mean soil temperature on each day and corrected for the diurnal temperature amplitude (Ågren *et al.*, 1980). Mean soil temperature data were gap-filled with averages of the temperatures recorded by functioning sensors from the same plot, cover type, and treatment. If these were not available, temperatures from drought plots were substituted for temperatures in control plots on the same aspect, and vice versa. The same was done for soil temperatures in ambient and irrigated plots.

After interpolation and correction for temperature, we had estimated annual total soil C efflux for each cover type within a plot, with 3 plots per treatment. We averaged the three totals within each treatment, yielding mean annual total soil C efflux for open areas, pinyon cover, and, in 2009, juniper cover, in the ambient, control, drought, and irrigated treatment. Juniper and pinyon were combined in 2007 and 2008 because we did not have Q_{10} values for juniper for those years. The time period included in the annual data is from day of year (DOY) 34 to DOY 352 because we wanted to compare the same

time period in each year and could not interpolate before the first measurement of 2007 or after the last measurement of 2009.

Analysis

Mean flux values in all treatments, including ambient, decreased from 2007 to 2009. We therefore compared effects of treatment and cover type within year. Attempts to model the data with parametric methods revealed violations of the assumptions of independent and identically distributed residuals and the data were highly non-normal. We used the Kruskal-Wallis test to identify significant differences among treatments and cover types within a year. If the test returned $P < 0.05$, a post-hoc multiple comparison test was used to identify which treatment pairs differed significantly. Differences in environmental variables were assessed with analysis of variance followed by the Tukey test of highly significant differences for normal data. Non-normal data were analyzed with the Mann-Whitney-Wilcoxon test or the Kruskal-Wallis test. All statistical analyses were done in R 2.11.1 (R Core Development Team, 2010), with the TINN-R interface.

Results

Environmental variables

Mean air temperatures remained steady during the study while precipitation declined each year. Total precipitation in 2009 was 82% of precipitation in 2007 (Table 2.1). The distribution of precipitation also changed. Rainfall in March and April of 2007 totaled 69 mm compared to 16 mm and 20 mm during the same months in 2008 and 2009, respectively (Fig. 2.1). Rainfall was more frequent and intense in late summer and early

fall of 2008 compared to 2007 and 2009 (Fig. 2.1). Three applications of water to the irrigated plots in 2008 totaled 60 mm, an 18% supplement to that year's precipitation. Irrigation was applied four times in 2009 and totaled 74 mm, a 24% addition. Since precipitation was declining over the time period (Table 2.1), irrigation treatments in 2008 and 2009 kept the annual totals in the irrigated plots near the precipitation levels of 2007.

The troughs succeeded in creating a moisture deficit in the drought plots (Fig. 2.2). Mean soil moisture in 2008 was significantly less in the drought treatment compared to the other treatments (Fig. 2.2). Irrigation was associated with increased soil moisture in 2009, but not in 2008 (Fig. 2.2). Soil moisture declined in the canopy locations in the ambient plots from 2008 to 2009, concurrent with the decrease in precipitation; soil moisture increased in the open locations at the same time (Fig. 2.2). The soil moisture decrease in the control plots in both canopy and open locations is consistent with the decrease in precipitation during the same time period. Soil moisture did not differ significantly among the ambient, control, and irrigation treatments during the growing season in 2008 but differed among all treatments during the growing season in 2009 (Table 2.2).

Soil temperatures in the two covered treatments, the drought and the control, were warmer by 2° – 4°C than in the uncovered treatments (Table 2.3) and did not differ significantly from each other (Table 2.4). Temperatures in the two uncovered treatments, (Table 2.3) also did not differ significantly from each other (Table 2.4) Mean annual soil temperatures in the ambient plot changed slightly from 2008 to 2009, with an increase of 0.2°C under tree canopies (Table 2.3). Mean soil temperature was 2.2°C warmer under trees in the drought plots compared to the ambient plots, and 1.8°C warmer in the open

(Table 2.3). Our experiment was intended to simulate the effects of changes in the amount of annual rainfall on soil CO₂ efflux. The treatment infrastructure warmed the soil, however, allowing us to compare the effects of the warmer drier climate in the drought treatments to those of the warmer climate in the control treatments. Effectively, the treatments were ambient, ambient temperature with added rainfall (irrigation treatment), warmer temperature with ambient rainfall (control treatment), and warmer temperature with decreased rainfall (drought treatment).

All mature pinyon trees in two of the three drought plots had died of insects or disease by 2009 (*personal observation*). Multiple pinyon trees also died in two of the irrigated plots and in one ambient plot. Herbaceous topgrowth essentially ceased in all drought plots by 2009; peak standing herbaceous crop in the drought plots in 2009 averaged only 5 g m⁻² (Table 2.5). The condition of the root systems was unknown. Junipers in the drought plots started dropping needles in 2009 (*pers. obs.*).

Soil CO₂ efflux

Soil CO₂ efflux generally followed winter-summer temperature patterns, with a precipitation spike in the spring and fall (2.1). Mean total soil C efflux ranged from 134 g C m⁻² y⁻¹ in the open areas of the drought plots in 2009 to 919 g C m⁻² y⁻¹ under pinyon canopy in an irrigation plot in 2007, before irrigation started (Fig. 2.3). The largest apparent effect on efflux values was related to the natural precipitation patterns in 2007. The wet spring in 2007 was associated with high soil CO₂ efflux compared to the same time of year in 2008 and 2009 (Fig. 2.1). Annual efflux rates declined in all treatments after 2007 (Fig. 2.3). The decrease was greatest in the drought treatment and similar

across the ambient, control, and irrigated treatments. The 18% decline in precipitation from 2007 to 2009 is similar to the 18% supplement to the irrigation plots in 2009 yet efflux values in the irrigation plots did not recover to the rates of 2007.

Differences among annual soil C efflux values across combinations of treatments and cover types within years were not statistically significant (all $P > 0.05$) except for the comparison between open areas in drought and control treatments in 2009 (Kruskal-Wallis critical test statistic = 19.86; observed = 21.1). Differences among annual soil C efflux values were significant between open space and pinyon canopy locations when tested across all years and treatments (Kruskal-Wallis critical test statistic = 13.8; observed = 19.8). When the tests were conducted within treatments, the result was the same except for the control treatment; there were no significant differences among fluxes according to cover type in the control treatment ($P = 0.96$). At the end of 2009, annual soil CO₂ efflux under trees was ordered by precipitation, with the lowest efflux in the warm, dry treatments (drought) and the highest in the irrigated treatments (Fig. 2.3). In the open areas, in contrast, the highest levels were in the control treatments, with ambient precipitation and increased temperature (Fig. 2.3).

Data from autochambers in open spaces provided a better fit to equation 1 than data from under tree canopies. The average r^2 of models fit to data from the irrigated, open autochamber was 0.71; the average r^2 from models fit to data from the autochamber under tree canopy in the same plot was 0.45. Similarly, the average r^2 for models using data from the open autochamber in the ambient plot was 0.83; the average r^2 from the canopy autochamber in the same plot was 0.31. Q_{10} values ranged from a low of 1.38 under pinyon in the control plots to a high of 2.82 under juniper in the irrigated plots

(Table 2.6). Q_{10} values were higher under pinyon trees than in open spaces in all treatments except the control (Table 2.6). Q_{10} values were highest under juniper (Table 2.6).

Soil C inputs

Litterfall in 2007 ranged from 400 g C m⁻² yr⁻¹ in the irrigated plots to 481 g C m⁻² yr⁻¹ in the control plots (Table 2.7). The range of values increased in 2009, from 490 g C m⁻² yr⁻¹ in the ambient treatment to 764 g C m⁻² yr⁻¹ in the drought treatment (Table 2.7).

Differences among treatments were not statistically significant (all $P > 0.45$). The net C flux for trees – annual litterfall C minus annual soil efflux C - was negative for all treatments in 2007 and positive for all treatments in 2009 (Table 2.7).

Discussion

The largest apparent effect on soil C efflux values was related to the natural precipitation patterns in 2007. The wet spring in 2007 was associated with high soil CO₂ efflux compared to the same time of year in 2008 and 2009 (Fig. 2.1). The higher total annual rainfall in 2007 was attributable mostly to the wet spring, coinciding with the start of the growing season. In contrast, the supplemental irrigation was delivered in small increments; none of the irrigation supplements in 2008 was applied at the beginning of the growing season and one supplement in 2009 was applied on DOY 301, after senescence of the understory vegetation. Given this natural experiment, where the decline in rainfall approximately equaled the irrigation supplement in the following years, we believe the difference in efflux rates over time, particularly in the ambient and

irrigated plots, reinforces the importance of the distribution of rainfall on ecosystem processes (*e.g.*, Knapp *et al.*, 2002).

The decline or death of vegetation in the drought treatment provided an opportunity for assessing the role of current photosynthate in supporting soil CO₂ efflux. The drought treatment was associated with the death of all mature pinyon trees and the understory yet soil CO₂ efflux near the trees did not decline to a level that differed significantly from the ambient or irrigated treatments; the diurnal flux pattern also continued unabated (Fig. 2.4). Either soil CO₂ efflux did not have the dependence on recent assimilate documented in some systems (*e.g.*, Tang *et al.*, 2005, Högberg *et al.*, 2009) or a change in the quantity or source of substrate offset the loss of fresh carbon inputs. Tree mortality caused a large increase in litterfall, increasing substrate availability. At the same time, the loss of protective tree canopies exposed fresh and accumulated litter to greater diurnal variability in temperature and soil moisture and increased the wetting effect of rains. Basal respiration levels in the drought plots were quite low but, as shown by the autochamber data, the newly opened canopy and deeper fresh litter layer produced a spike in soil CO₂ efflux that exceeded the response to rainfall in the other treatments (Fig. 2.4). We do not know how much of the spike was caused by physical diffusion from the porous litter layer and how much was biogenic. The fact that the response lasted for several days, however, suggests a rapid sustained heterotrophic response to moisture inputs. A similar rainfall response did not occur in the open spaces that had had grass cover at the start of the experiment. We assume the soil in these open spaces contained dead or dying fine roots; perhaps the more labile carbon was rapidly

consumed and there was not a replacement substrate, such as the litterfall from the tree canopies.

Given the greater values for litterfall inputs than for soil carbon efflux, we estimate that the drought plots are accumulating soil carbon on an annual basis. The death of the pinyon, the cessation of topgrowth in the herbaceous layer, and the declining condition of the juniper indicate minimal belowground carbon allocation. This might be a relatively short-term effect since no significant additional litter inputs are expected and litter under newly opened canopies might be scattered by the wind over time or decompose more rapidly in the new moister environment resulting from the lack of canopy cover. We do not know how much belowground allocation occurred in the non-droughted plots. The irrigation treatments, warmer temperatures in the control plots, and large inter-annual variability in rainfall argue against a steady-state assumption. The difference between source and sink is likely to be determined by moisture availability, as suggested by the source status of the wooded component in all plots during the wet year of 2007 (Table 2.7), followed by a switch to sink status in the succeeding years; we are defining source and sink based solely on the difference between litterfall and respiration. The switching pattern suggests moisture limitation in the organic horizons during drought years (Borken *et al.*, 2006), with eventual mineralization after the resumption of rainfall. Increases in soil organic horizons with decreased precipitation have been observed previously in some, but not all, artificial droughts in forests (*e.g.*, Fröberg *et al.*, 2008, Ozolinčius *et al.*, 2009). The increase in litterfall in the control treatments was not caused by tree death and we cannot assign a cause to it. We do not know of a relationship between higher soil temperatures and faster leaf turnover. Pinyon roots

might stop functioning at high soil temperatures (Williams *et al.*, 2000) which, we speculate, could accelerate leaf turn-over rates.

Increasing soil moisture in the drought treatments from 2008 to 2009 (Fig. 2.2) is also likely to have been related to the decline or death of the overstory and understory vegetation. Throughfall should have increased with the loss of the tree canopy and transpiration would have decreased. We did not determine the extent of the root systems in either the overstory or the understory. Given the shallow soils and the age of the trees, however, we believe the tree roots would have extended far beyond the drip line of the tree canopies. The physiological decline of the overstory and understory vegetation might have been too advanced or too simultaneous for individual plants to take advantage of the decreased competition for water resources. Qualitatively, we observed an increase in forbs below the former tree canopies.

Comparison to other sites

Annual values for soil CO₂-C efflux at our site were comparable in magnitude and inter-annual variability to those in a seasonally moisture-limited ponderosa pine forest where the mean value was 811 g C m⁻² yr⁻¹ and the range was 427 g C m⁻² yr⁻¹ (Irvine *et al.*, 2008). Soil efflux rates at our site were lower compared to more northerly semi-arid grassland (1,022 g C m⁻² yr⁻¹; (Frank *et al.*, 2006) or a more southerly subtropical savanna (12.7 g C m⁻² day⁻¹ for woody vegetation and 9.8 g C m⁻² day⁻¹ for grassland; McCulley *et al.*, 2007). Values were comparable to annual efflux rates from various sites around the globe (Singh *et al.*, 1977; Raich *et al.*, 1992; Bahn *et al.*, 2010) but not with a specific vegetation type.

The Q_{10} values for pinyon canopy locations and open spaces were quite low (Raich *et al.*, 1992; Conant *et al.*, 2004), possibly indicating the importance of soil moisture in driving respiration rates in semi-arid ecosystems. We estimated Q_{10} when soil moisture was not changing which, in semi-arid systems with loose sandy soils, is generally when soils are dry. Moisture limitation would inhibit a strong response to temperature, resulting in a low Q_{10} (Bryla *et al.*, 1997; Conant *et al.*, 2004).

Alternatively, the low Q_{10} might reflect a nutrient limitation on respiration; Q_{10} was much higher under juniper than under pinyon. Juniper litter also has a lower C:N ratio than pinyon litter (Klopatek *et al.*, 1998), potentially an important factor in this nitrogen-limited location. In the irrigated plots, the Q_{10} under pinyon and juniper were similar, suggesting a third explanation. Juniper-derived organic matter (OM) could have better moisture holding capacity than pinyon-derived OM, reducing the moisture limitation suggested in our first explanation. Irrigation treatments might erase this limitation in pinyon-derived OM, equalizing the respiration response to temperature in litter of the two species.

Sources of error

Daily values for soil CO₂ efflux were interpolated with a temperature-based model. The interpolated values include seasonal soil moisture patterns because we projected between monthly measurements made at the collars. Short-term increases in efflux associated with changes in soil moisture were not captured so our estimated values are probably low. The extent of the underestimate depends on the relative contributions of the pulse-driven efflux and seasonal moisture patterns with respect to the annual total and on the

previously mentioned low Q_{10} values (Yuste *et al.*, 2005). The greatest sampling intensity was in the wooded parts of the plots; most of the area was herbaceous. Pinyon was sampled more intensively than juniper; juniper was the dominant tree species. The greater sampling intensity in the less dominant vegetation suggests that the variance estimates may be more precise for the trees than the understory, and more precise for the pinyon than the juniper.

Conclusion

At our semi-arid study site, changes in moisture availability did not create differences in annual soil CO₂ efflux as large as those between pinyon pine and grass. Changes in rainfall regimes are likely to be associated with changing percentages of trees, shrubs, grasses, succulents, or exposed soil (Anderson 1991; Stampfli *et al.*, 2004; Williams *et al.*, 2007), with implications for soil CO₂ efflux rates, C storage potential, and erosion. These structural changes may lead to unexpected mid-term results, such as the increasing soil moisture in the drought treatments. Dying vegetation will leave legacies, such as the litter at our study site, that can initially drive ecosystem processes, yet gradually fade in importance. The overstory and understory mortality at our study site was not a confounding factor. It was a reminder that predicting ecosystem response to changing precipitation regimes goes beyond soil moisture content, potential evapotranspiration rates, or hydraulic conductivity and extends into the relationship between ecological structure or function and water and nutrient cycles.

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Table 2.1. Mean (SE) annual climate variables and total precipitation

Year	PAR $\mu\text{mol m}^{-2} \text{sec}^{-1}$		Temperature ($^{\circ}\text{C}$)		VPD (kPa)		Precipitation (mm)
2007	415	(8)	13	(0.5)	1.09	(0.7)	377
2008	433	(8)	13	(0.4)	1.12	(0.7)	331
2009	412	(8)	13	(0.4)	1.16	(0.7)	310

Table 2.2. *P*-values for pairwise comparison of soil moisture averaged across treatments within year and location. Comparisons were made for the entire year and the growing season (DOY 90-270). A-Ambient, C-Control, D-Drought, I-Irrigated.

Location	Year	Treatment comparisons					
		C-A	D-A	I-A	D-C	I-C	I-D
Open	2008	0.51	0.00	0.01	0.00	0.27	0.00
	DOY 90 to 270	1.00	0.00	0.70	0.00	0.70	0.00
	2009	0.00	0.00	0.00	0.00	0.00	0.00
	DOY 90 to 270	0.00	0.00	0.00	0.00	0.00	0.00
Canopy	2008	0.84	0.00	0.31	0.00	0.05	0.00
	DOY 90 to 270	0.81	0.00	0.83	0.00	0.30	0.00
	2009	0.18	0.00	0.00	0.00	0.00	0.00
	DOY 90 to 270	0.00	0	0	0	0	0

Table 2.3. Mean (SE) annual soil temperature by treatment and location within year.

Location	Year	Treatment							
		Ambient		Control		Drought		Irrigated	
Open	2008	18.4	(0.49)	20.1	(0.50)	20.8	(0.51)	16.8	(0.48)
	2009	18.6	(0.54)	20.5	(0.53)	20.4	(0.53)	16.6	(0.49)
Canopy	2008	14.4	(0.36)	15.8	(0.37)	16.4	(0.38)	14.1	(0.35)
	2009	14.6	(0.37)	16.4	(0.39)	16.8	(0.40)	14.4	(0.38)

Table 2.4. *P*-values for pairwise comparisons of soil temperature averaged across treatments within year and location. A-Ambient, C-Control, D-Drought, I-Irrigated.

Location	Year	C-A	D-A	I-A	D-C	I-C	I-D
Open	2008	0.10	0.01	0.16	0.82	0.00	0.00
	2009	0.07	0.07	0.08	1.00	0.00	0.00
Canopy	2008	0.07	0.00	0.93	0.81	0.01	0.00
	2009	0.01	0.00	0.99	0.96	0.00	0.00

Table 2.5. Mean (SE) aboveground net primary production of the herbaceous understory in 2009. Data were not collected in control plots.

Treatment	ANPP (g m ⁻² yr ⁻¹)	
Ambient	50	1
Control	n.d.	n.d.
Drought	5	3
Irrigated	51	10

Table 2.6. Estimated Q_{10} values and the coefficient of determination (r^2) by species and treatment. Q_{10} was not estimated for juniper in the control treatment.

Location	Treatment		Control	r^2	Drought	r^2	Irrigated	r^2
	Ambient	r^2						
Open	1.63	0.83	1.92	0.57	1.37	0.57	1.86	0.71
Pinyon	1.76	0.31	1.38	0.05	1.73	0.57	2.41	0.45
Juniper	2.17	0.44	n.d.	n.d.	2.62	0.33	2.82	0.54

Table 2.7. Mean (CV) annual litterfall ($\text{g C m}^{-2} \text{ yr}^{-1}$) and the difference between litterfall and soil $\text{CO}_2\text{-C}$ efflux ($\text{g C m}^{-2} \text{ yr}^{-1}$) by treatment in 2007 and 2009.

	2007		2009	
Litterfall				
Ambient	473	(0.36)	490	(0.12)
Control	481	(0.04)	618	(0.32)
Drought	451	(0.23)	764	(0.98)
Irrigated	400	(0.16)	517	(0.15)
Litterfall minus respiration				
Ambient	-102		92	
Control	-106		227	
Drought	-311		478	
Irrigated	-519		86	

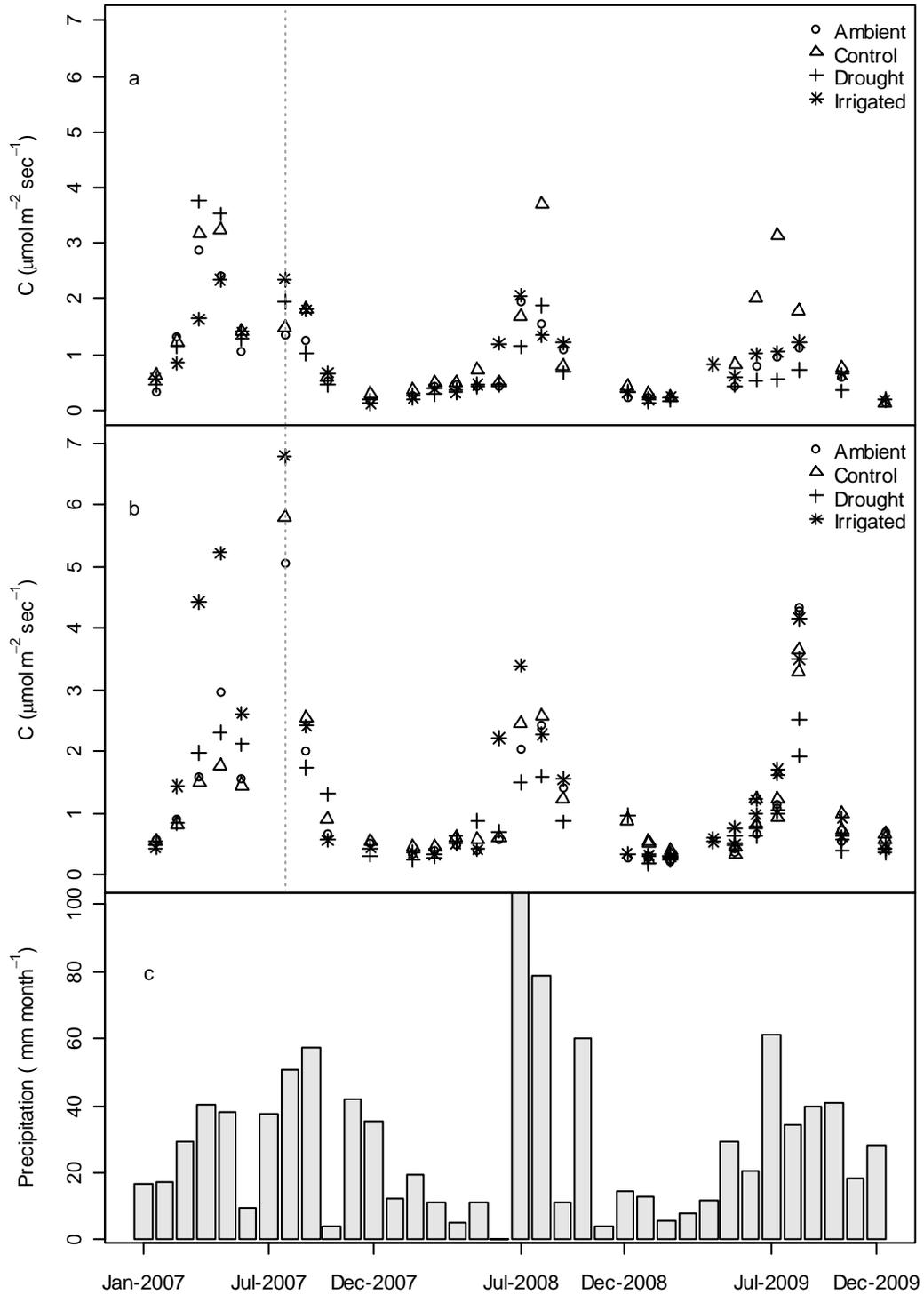


Fig. 2.1. Mean soil CO₂-C efflux measured at the collars: a- open locations; b-canopy locations. Values are standardized to the mean temperature on the day of sampling, without correction for diurnal temperature amplitude. Vertical line indicates approximate date when the installation of covers was complete.

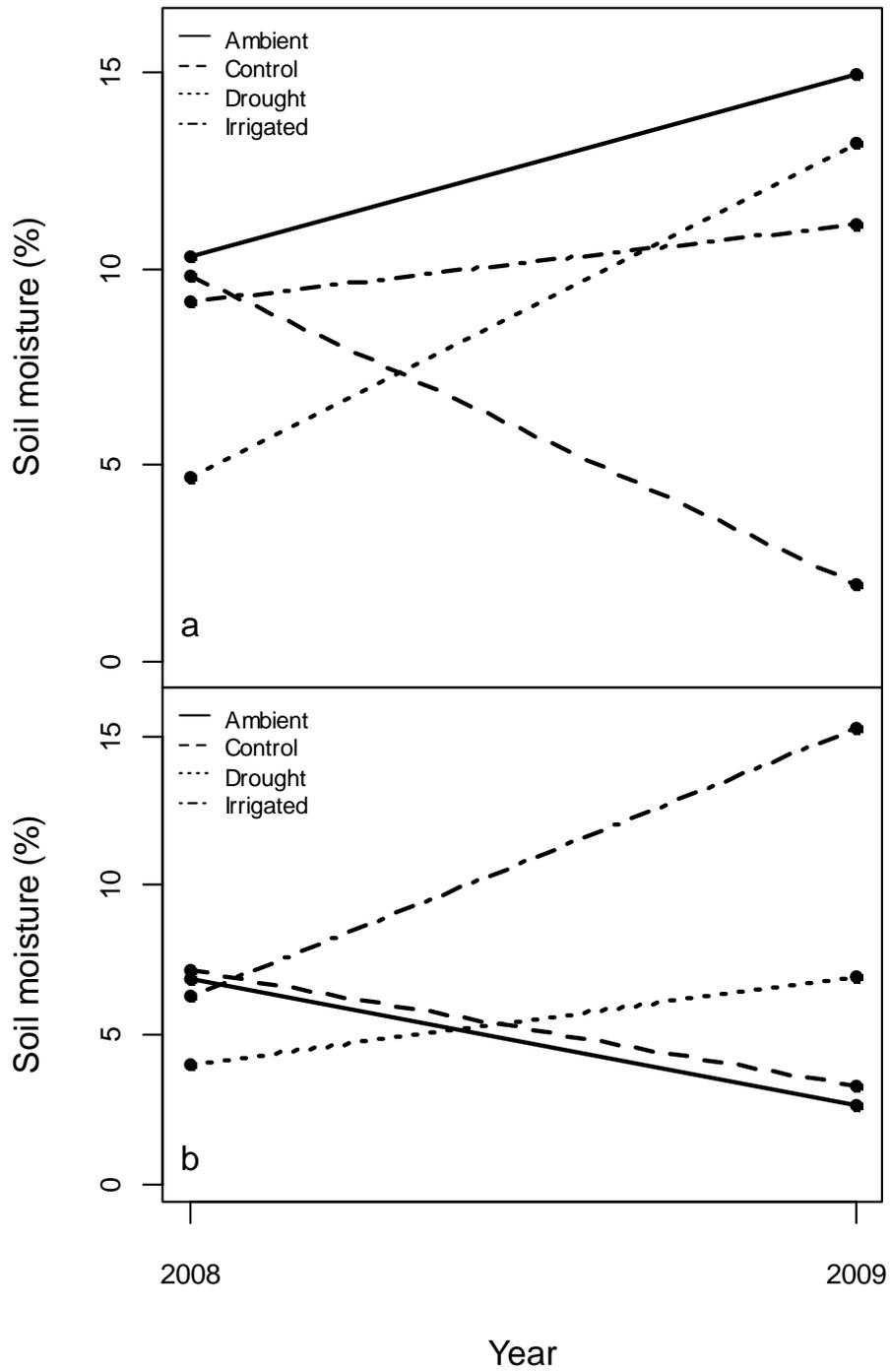


Fig. 2.2. Mean annual soil moisture (%) by treatment and year in a) the open locations; and b) under tree canopies. Error bars are two standard errors.

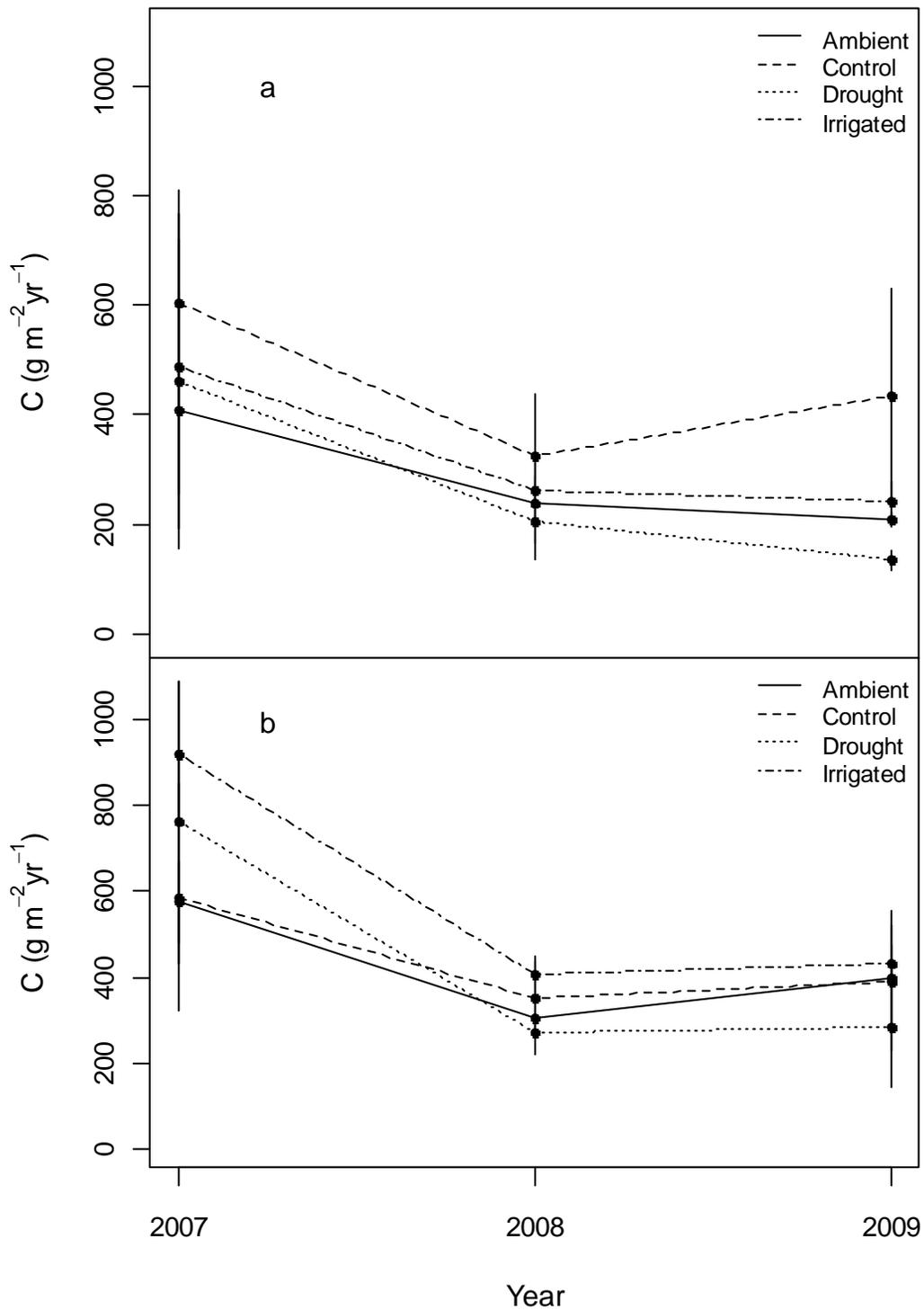


Fig. 2.3. Mean annual soil CO₂-C efflux by treatment and year: a-open locations; b-under canopies. Error bars are two standard errors of the mean. The only significant within-year differences ($p < 0.05$) are between the drought and control locations in panel a.

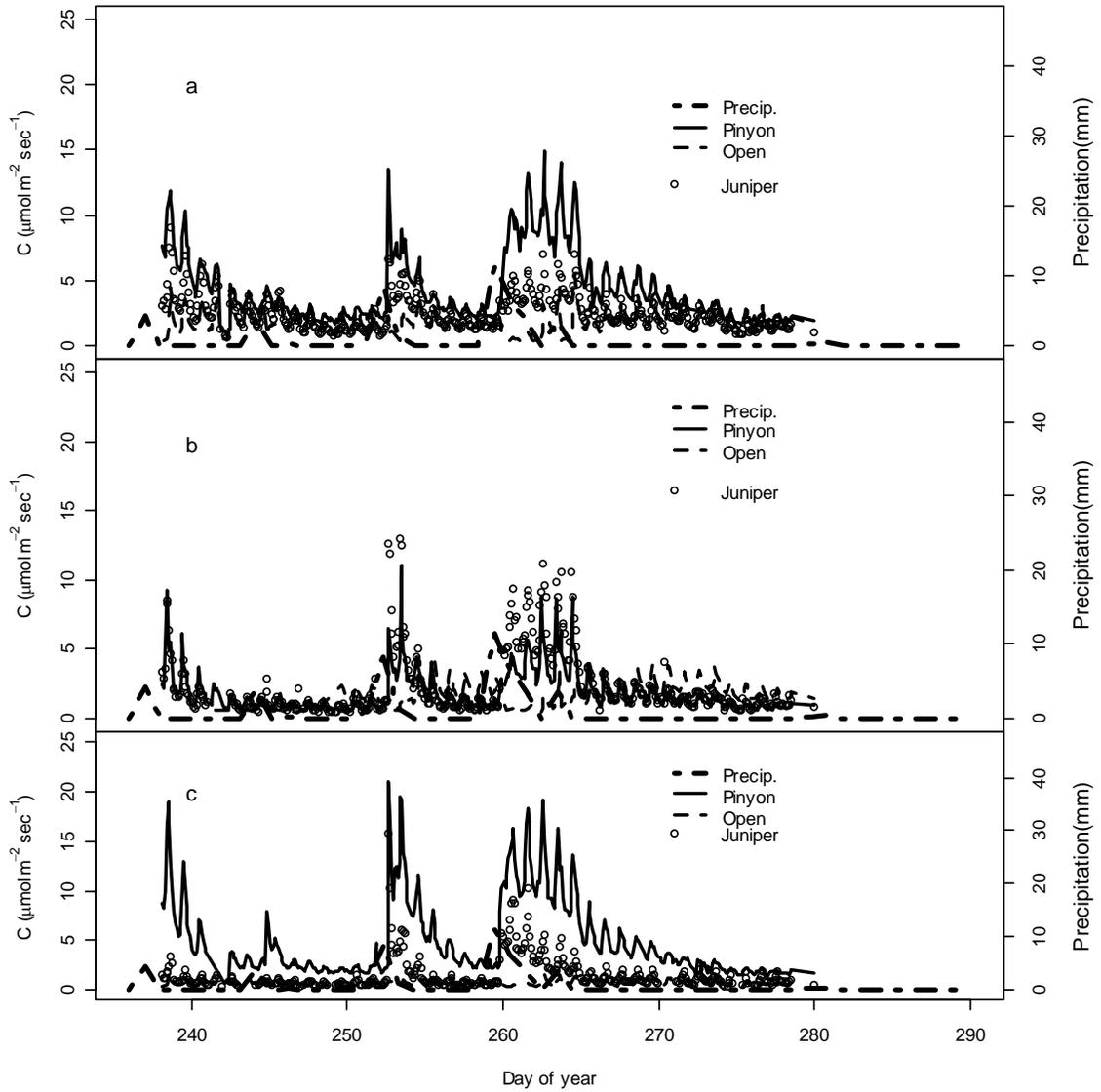


Fig. 2.4. Autochamber time series for 45 days in 2009: a) Ambient; b) Irrigated, with irrigation applied on DOY 235; and c) Drought. The pinyon tree in panel c was dead. Data were recorded every 2 hours. Understory vegetation senesced near the end of the period.

Table S1. Spot collar sampling frequency and intensity by month and year. A complete sample is 12 plots with 9 collars per plot.

	2006	2007	2008	2009
Jan		No sample	No sample	12 plots 9 collars per plot
Feb		9 collars sampled	12 plots 6 collars per plot	12 plots 9 collars per plot
March		12 plots 9 collars per plot	12 plots 6 collars per plot	No sample
April		12 plots 9 collars per plot	12 plots 6 collars per plot	1 plot 8 collars
May		12 plots 9 collars per plot	12 plots 6 collars per plot	12 plots 9 collars per plot
June		12 plots 9 collars per plot	12 plots 6 collars per plot	12 plots 9 collars per plot
July		No sample	12 plots 6 collars per plot	12 plots 9 collars per plot
August		8 plots 9 collars per plot	12 plots 6 collars per plot	12 plots 9 collars per plot
September		12 plots 6 collars per plot	12 plots 9 collars per plot	12 plots 9 collars per plot
October	12 plots 9 collars per plot	6 plots 6 collars per plot	No sample	12 plots 9 collars per plot
November	12 plots 9 collars per plot	No sample	No sample	No sample
December	12 plots 9 collars per plot	4 plots 6 collars per plot	8 plots 9 collars per plot	12 plots 9 collars per plot

CHAPTER 3

Soil nutrient losses in an altered ecosystem are associated with native ungulate grazing

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Summary

1. Protected natural areas often inherit a legacy of altered habitat and predator-prey dynamics. Classical models of plant-herbivore-nutrient cycles may not apply in these non-equilibrium conditions or when the physical capacity of the soil to process nutrients is altered.
2. Our objective was to examine the association between intensive grazing by *Cervus elaphus* (elk or red deer) and soil processes in a protected natural area with a history of predator reduction and habitat alteration. We used 12 exclosures constructed in 1994 to compare soil carbon and nitrogen, nitrogen cycling, bulk density, soil moisture, and soil microbial structure and function in grazed and ungrazed areas. Samples were collected in 1998 and in 2010.
3. Soil carbon concentration was 25% lower in grazed areas than in exclosures; soil nitrogen concentration was 23% lower. Soil carbon and nitrogen did not increase in the exclosures between 1998 and 2010. Soil carbon and nitrogen decreased in grazed areas during the same time period.
4. Soil bulk density in grazed areas was 25% greater than in ungrazed areas and soil moisture in grazed areas was 15% lower than in ungrazed areas.
5. Physical soil attributes, soil nutrient stocks, and soil microbial biomass were significantly correlated, exhibiting linkages that can accelerate changes in ecosystem function.
6. Synthesis and applications. Consumption patterns and spatio-temporal distributions of ungulates are associated with potential changes in ecosystem structure and function as a

consequence of changes in soil structure and nutrient loss. The density and distribution of the elk populations should be controlled in the short term to restore or protect soil structure, hydraulic conductivity, and nutrient content. Restoration of landscape structure or predator populations might alleviate the need for active management of the elk population in the long term.

Key-words: bulk density, *Cervus elaphus*, EL-FAME, natural areas, Rocky Mountain National Park, *Salix*, soil carbon, soil compaction, soil nitrogen

Introduction

Cervus elaphus (elk or red deer), a large grazing animal formerly found throughout North America, was hunted nearly to extinction in the 19th century (Committee on ungulate management in Yellowstone National Park, 2002). Regulatory reforms such as prohibitions on market hunting (18 United States Code 42-43 and 18 United States Code 3371-3378) and policy changes in federally protected natural areas reinvigorated elk populations in the western United States during the 20th century (Committee on ungulate management in Yellowstone National Park 2002). Simultaneously, predator populations were reduced or locally extirpated and habitat was altered by changes as disparate as the construction of irrigated landscapes and the decline of *Castor canadensis* (beaver) (Committee on ungulate management in Yellowstone National Park 2002). The rebound in *C. elaphus* populations prompted concerns about overgrazing, particularly in protected natural areas such as Rocky Mountain National Park and Yellowstone National Park (Singer & Schoenecker 2003). Determining historical population levels against a backdrop of human-caused population extirpation, subsequent regulatory intervention, and habitat alteration is probably not possible (Committee on ungulate management in Yellowstone National Park 2002). A reasonable objective is to determine whether or not the distribution of *C. elaphus* is associated with ecosystem changes that might signal impending alteration or degradation.

Our research focuses on the response of soil structure, nutrient cycling, and microbial ecology to *C. elaphus* use of riparian zones during winter and spring. Studies of grazer effects on nutrient cycling are legion and have been reviewed elsewhere (*e.g.* Singer & Schoenecker 2003; Harrison & Bardgett 2004). Few studies on herbivory and

nutrient cycling incorporate soil physical properties such as compaction or soil moisture. Moisture availability is a key component, however, of several models that predict ecological responses to grazing (e.g., McNaughton 1979, Parton *et al.* 1987). A process model of soil organic matter in grasslands identified soil texture and moisture as important determinants of organic matter retention (Parton *et al.* 1987). Soil water potential also contributes to the increase in production associated with the grazing-optimization hypothesis (McNaughton 1979). Soil moisture and bulk density can affect the rates of nutrient cycles, independent of the quality or quantity of litter inputs (Powers *et al.* 2005). Theoretical models of the relationship between grazing and nutrient cycling include those in which rates matter (e.g., Loreau 1995) and those in which rates do not matter (e.g., Mazancourt *et al.* 1998); the explicit equilibrium assumption of both types of models might not be applicable when dominant herbivores and their predators are alternately extirpated and protected, as is the case in our study area.

Ecosystem N budgets calculated in the late 1990's indicated that grazed riparian zones in Rocky Mountain National Park were losing nitrogen (N) at the rate of 1.8 g N m⁻² yr⁻¹ (Schoenecker *et al.* 2004). Soil N levels did not differ between grazed and ungrazed areas; N mineralization rates differed in some plots but not in others (Schoenecker *et al.* 2004). Elsewhere in the park, *C. elaphus* grazing was associated with an increase in soil bulk density (Binkley *et al.* 2003). We returned to the original research area 16 years after installation of grazing exclosures and 12 years after collecting preliminary data to study the relationship between *C. elaphus* grazing and soil processes. We hypothesized that ungrazed areas would have lower bulk density, higher nutrient levels, greater microbial biomass, and faster nutrient cycles, as indicated by net

N mineralization, than grazed areas. We expected the changes to reflect increases, or recovery, in the ungrazed areas, rather than losses from the ungrazed areas. We also expected microbial community composition to differ between grazed and ungrazed areas due to differences in aboveground vegetation and to the abundant N inputs from *C. elaphus* in grazed areas.

Our study is one of few to explore the effects of native ungulate grazing on soil processes. More often, ungulate grazing is evaluated with respect to changes in plant community composition. This focus can belie changes in soil function that are difficult to reverse, with implications for ecosystem sustainability (Milchunas & Lauenroth 1993). The issues we raise are applicable in many regions of the world where predator reduction and habitat modification have altered the ecological dynamics of native grazers.

Methods and Materials

The study sites are in riparian zones of parallel glacial valleys, Moraine Park and Horseshoe Park, in Rocky Mountain National Park, Colorado, USA. The sites are at an elevation of approximately 2,500 m; the latitude and longitude are 40.4° N and 105.6° W. Mean annual air temperature ranges from 2°C to 6°C. The annual frost-free period varies from 40-75 days. Mean annual precipitation in the nearby village of Estes Park (elev. 2293 m) is 350 mm. The majority of the annual precipitation falls between April and September. Soils in the two valleys are coarse-loamy nonacid Fluvaquentic Cryaquepts in the Kawuneeche series (Soil Survey Staff 2007). Mineral soil material is alluvium over sandy and gravelly glaciofluvial deposits derived from granite, gneiss, and schist (Soil Survey Staff 2007). Soil texture to 15 cm ranges from loam and sandy loam to

mucky peat over clay loam (Soil Survey Staff 2007). Mean summer soil temperature is approximately 6°C and soils are frequently flooded in spring and summer (Soil Survey Staff 2007). Both valleys were used for human activities ranging from homesteading to golf courses from the mid 19th century through the mid 20th century. The extent of Native American use of the valleys is uncertain (Brett 2003).

Horseshoe and Moraine Parks are part of the winter range of the Rocky Mountain National Park herd of *C. elaphus*. Winter *C. elaphus* density in Moraine Park averaged 30-65 animals km⁻² from 1994-1999 (Singer 2002). Vegetation in both valleys includes *Salix monticola*, *Salix planifolia*, *Salix geyeriana* (willows), *Betula occidentalis* Hook. (water birch), *Betula glandulosa* (dwarf birch), *Potentilla fruticosa* (shrubby cinquefoil), *Carex* spp. (sedges), *Juncaceae* (rushes), *Gramineae* (grasses), and flowering plants. Wintering *C. elaphus* consumed approximately 31% of annual *Salix* production in Moraine Park and 22% in Horseshoe Park between 1994 and 1999 (Singer *et al.* 2002).

Concerns about changes in riparian plant communities, including loss of *Salix*, prompted construction of 30.5 m x 45.7 m grazing exclosures in 1994 (Singer & Zeigenfuss 2002). The 2.4 m high fences were designed to exclude large ungulates but not small herbivores such as *Sylvilagus* spp. (cottontail rabbits) and *Lepus* spp. (hare) (Schoenecker *et al.* 2004). Each exclosure was paired with a nearby grazed study site of the same size; each exclosure and grazed area constitute a paired plot. Six paired plots were located in Moraine Park and six in Horseshoe Park. Four plots in each valley represented a vegetation type known as “short willow”, a local morphological classification correlated with heavier *C. elaphus* browsing and a lack of current *C. canadensis* activity (Zeigenfuss *et al.* 2002). Water levels in two of the four “short

willow” plots in each valley were artificially raised to simulate higher water tables associated with beaver presence. The two remaining plots in each valley were in the “tall willow” vegetation type and were approximately 1 - 2 km from the “short willow” plots.

SAMPLING AND LABORATORY ANALYSES

The exclosures were divided into four quadrants during the 1990’s. Three quadrants received treatments such as burning or fertilization and the fourth was a control. We sampled soils from the control quadrant of the exclosure and from one quadrant in the ungrazed area of each paired plot in 1998 and 2010. We could not resample the ungrazed area of two plots in Horseshoe Park in 2010 because they had been incorporated into newer exclosures. We therefore substituted a grazed area outside the fence surrounding the original grazed plots.

We assessed plant-available N during July and August of 1998 and 2010 using closed-top cores (Adams & Attiwill 1986) to estimate net mineralization. In 1998, six PVC tubes were inserted 15 cm into the mineral soil along two parallel transects of 3 tubes each. Spacing was 7 m between tubes within a transect and 5 m between transects. The tubes were capped and incubated in the field for 6-7 weeks. A second tube was inserted at every other sampling location and removed immediately for assessment of the initial N concentrations. In 2010, three pairs of tubes were inserted 19 cm deep along a location approximating one of the original transects. One tube was immediately removed and the remaining tube was capped and incubated in the field for 6-7 weeks. Collected tubes were stored in a cooler and taken to the laboratory at the end of each day. The three initial and incubated subsamples from each plot were weighed, composited and sifted

through a 4 mm or 4.75 mm sieve within 24 hours of arriving at the laboratory. We extracted 10 g of the composited sifted soil from each plot with 50 ml of 2 M KCl. The amount of ammonium-N and nitrate-N in the KCl solution was determined colorimetrically on a Perstorp automated colorimeter in 1998 and on an OI Flow Solution IV in 2010 (OI Analytical Products, College Station, Texas, USA). Net mineralization was estimated by subtracting the values derived from the initial samples from those of the incubated samples and summing the two forms of N. An additional subsample from each plot was dried at 105°C for determination of soil moisture, solute dilution and, in 2010, bulk density. In 1998, bulk density was estimated from a separate set of samples collected along parallel transects on either side of the enclosure fence. Soil pH was measured on this set of samples.

Nitrogen availability was also evaluated using ion-exchange resin bags (Binkley & Matson 1983) to indicate relative availability of ammonium-N and nitrate-N. For the 1998 sampling, nylon resin bags were divided into two sections to hold the separate anion and cation beads (Sybron IONAC ASB-IPOH and IONAC c-251 H⁺, Birmingham, New Jersey, USA). Six bags were placed 2 cm below the mineral soil surface in each plot, along the same transects used for the field incubations. Mixed-bed ion exchange resins were used in 2010; three heat-sealed nylon bags were placed 2 cm below the mineral soil surface adjacent to the tubes used for the field incubations. Resin bags were installed and removed at the same time as the samples for the field incubations. The amount of ammonium-N and nitrate-N captured by the resins was analyzed in the same manner as and simultaneously with the field-incubated samples; resins were not composited by plot but were analyzed as separate samples.

We estimated total carbon (C) and N from ground oven-dried soil samples from the grazed and ungrazed plots. Soil samples were collected along parallel transects inside and outside the exclosures in 1998; in 2010, we used a subsample of the soil composited for the N-mineralization analysis. Total C and N were determined by dry combustion (LECO-1000 (1998) and LECO-TruSpec CHN (2010), LECO Corporation, St. Joseph, Michigan, USA).

We characterized soil microbial activity in 2010 from an additional set of samples collected in early July, 2010. We collected six soil cores from parallel transects in each plot, at approximately the same locations sampled in 1998; the tubes were 19 cm long. The samples were stored in a cooler and taken to the laboratory at the end of each day. Samples were composited by plot and sifted through 4 mm or 4.75 mm sieves in the laboratory. The composited samples were divided into subsamples for determination of microbial biomass, microbial community composition, and soil moisture content. The subsamples designated for analysis of community composition were stored at -80°C and extracted within one week of collection.

We estimated the amount of C and N in soil microbial biomass using chloroform fumigation-extraction (Brooks 1985, Vance 1987). One set of soil samples from each plot was extracted with 0.5 M K₂SO₄. A second set was extracted following 5 days of fumigation with ethanol-free chloroform in a vacuum dessicator. The difference between the starting and ending concentration of C and N in each sample is proportional to the amount of microbial biomass C and N in the dry soil samples. We used the conversion factor 0.54 (Brookes *et al.* 1985) for N and 0.45 for C (Vance *et al.* 1987) to estimate biomass N and C. Conversion factors are best developed for specific soils. Applying the

same factor to neighboring plots should not affect the comparisons that are our goal but the results might not accurately represent the actual biomass present.

Microbial community composition was assessed by extracting methylated ester-linked fatty acids (EL-FAME; Drijber, unpublished, described in Schutter & Dick 2000) from fresh soil samples. The fatty acid nonadecanoic acid (19:0) was added to each sample for calculation of relative mole percent of individual fatty acid biomarkers. The extractant was analyzed on an Agilent 6890 gas chromatograph (Agilent Technologies Inc., Palo Alto, California, USA) at the University of Delaware after addition of a hexane/methyl butyl tertiary ether solvent. Biomarkers were identified by MIDI Microbial ID software (Newark, Delaware, USA). We used EL-FAME 15:0, *a*15:0, *i*15:0, *i*15:1, *i*16:0, 17:0, *a*17:0, *i*17:0, 17:0 cy, 10Me17:0, 17:1 ω 8c, 10Me18:0, 19:0 cy, and C22 primary alcohol as indicators of bacterial communities. We used 18:2 ω 6c as an indicator of saprophytic or ectomycorrhizal fungal communities. EL-FAME's 16:1 ω 5c, 16:1 ω 7c, and 16:1 ω 9c can indicate bacteria or arbuscular mycorrhizal fungi (AMF). In this wet environment with potentially dual mycorrhizal *Salicaceae* (Newman & Reddell 1987, Cornwell *et al.* 2001), we were not sufficiently confident to assign either identity to these markers. Protozoa were represented by 20:3 ω 6c and 20:4 ω 6c (Cavigelli *et al.* 1995); these may equally be fungal biomarkers (Müller *et al.* 1994, Bossio & Scow 1998). We recognize that biomarkers are not exclusive to groups of soil microbes and based our classification on a synthesis of Cavigelli *et al.* (1995), Bossio & Scow (1998), Olsson (1999), Zelles (1999) and Ritchie *et al.* (2000).

STATISTICAL TESTS

The effects of grazing and location on the sampled soil attributes were determined with Student's t-test for paired samples when data were normal and with the Mann-Whitney-Wilcoxon rank correlation test when data were not normally distributed. Tests were one-tailed when addressing a hypothesis of greater or lesser effect and two-tailed otherwise. Correlations between measured attributes were assessed with least-squares regression. Although the structure of the data suggests the use of mixed-effects models, model comparisons indicated mixed-effects models provided no additional information and worsened model fit. When model diagnostics indicated violations of the assumption of normality, correlations were re-assessed with the Kendall rank correlation coefficient, τ . Statistical analyses were conducted in R 2.10.1 (R Core Development Team 2010) with a significance level of $\alpha = 0.05$.

We used hierarchical cluster analysis with complete linkages to group treatment plots according to similarities of EL-FAME's (Venables & Ripley 2002). Treatment effects on the relative mole percent of bacterial biomarkers were estimated with Student's t-test and with two measures of physiological stress, the ratio of 17:0 cy to 16:1 ω 7c and of 19:0 cyclo to 18:1 ω 7c (Grogan & Cronan 1997). We used non-metric multi-dimensional scaling followed by a permutation procedure (Oksanen *et al.* 2010) to quantify the relationship between microbial community structure and environmental variables, including the presence or absence of grazing. Goodness-of-fit was assessed on the basis of the root mean square error for the initial ordination. The fit of the environmental variables to the ordination axes was indicated by a correlation coefficient; the associated metric of statistical significance was generated by permutation analysis

(Oksanen *et al.* 2010). We used the Bray-Curtis algorithm for estimating dissimilarity and ran the permutation procedure 5000 times.

Results

Salix have become the dominant vegetation in the 16 years since exclosure construction (Fig. 3.1). Plot 1 has the characteristics of a true wetland and the grazed half was under approximately 9 cm of water during sampling for microbial biomass and community composition. Excluding plot 1, soil moisture in 2010 ranged from 17% to 51%; bulk density varied from 0.26 kg L⁻¹ to 1.01 kg L⁻¹ (see Table S1 in Supporting information). The range of most variables was wide, particularly when plot 1 was not excluded from calculations (see Tables S1 and S2). The highest C:N ratio was 21.3 in the grazed half of plot 2 and the lowest was 12.3 in the grazed half of plot 7. The range of values was similarly broad in 1998 (see Table S2). Visible soil characteristics differed; the light sandy soils of plots 2, 3, and 4 contrasted with the mucky peat soils of plot 1 (see Fig. S1). Most measured variables differed significantly between Horseshoe and Moraine Parks (Table 3.1).

PHYSICAL SOIL PROPERTIES

Soil bulk density was significantly greater in grazed plots compared to ungrazed plots in 1998; the difference was larger in 2010. Bulk density in grazed plots was 11% higher in grazed plots compared to ungrazed plots in 1998 and 35% higher in grazed plots compared to ungrazed plots in 2010 (Table 3.2). Soil moisture was higher in ungrazed compared to grazed plots in both years; the statistical difference was weaker in 2010 but

the differences in mean values was similar. Bulk density and soil moisture were significantly negatively correlated across years and treatments ($R^2 = 0.64$, $P \approx 0$; Fig. 3.2).

SOIL C AND N

Soil carbon (C) was similar in grazed and ungrazed plots in 1998 (Table 3.2). In 2010, soil carbon in grazed plots was 80% of that in ungrazed plots on a mass per mass basis. Soil N was also similar in ungrazed and grazed plots in 1998; in 2010, soil N in grazed plots was 77% of soil N in ungrazed plots (Table 3.2). Soil N and C in the grazed plots declined by 33% and 30%, respectively, over the course of the study. The C:N ratio did not differ between treatments in either year. Treatments appeared to affect the amount of nitrogen collected by the resins similarly in both years, with mean resin N approximately 50% lower in grazed plots compared to ungrazed plots (Table 3.2). Mean net N mineralization was 30% - 40% lower in grazed compared to ungrazed plots in each year; this difference was not statistically significant. Resin N and net mineralization were not correlated in 1998 ($P > 0.05$); they were weakly yet significantly correlated in 2010 ($R^2 = 0.28$, $P = 0.006$). Net N mineralization was significantly correlated with bulk soil N in 2010 ($R^2 = 0.55$, $P \approx 0$, slope = 0.43); plot 1 was not included in the model. Soil moisture declined an average of 13% ($P = 0.009$) during the closed-top incubation.

Net N mineralization was significantly but weakly correlated with soil moisture in 1998 ($R^2 = 0.31$, $P = 0.003$); in 2010, the relationship was not statistically significant ($P > 0.05$). Soil C was significantly correlated with soil moisture across both years and treatments ($R^2 = 0.64$, $P \approx 0$, Fig. 3.3). A similar relationship existed between soil N and

soil moisture ($R^2 = 0.52$, $P \approx 0$; Fig. 3.3). Soil C was significantly correlated with bulk density across years and treatments ($R^2 = 0.7$, $P \approx 0$) as was soil N ($R^2 = 0.62$, $P \approx 0$; Fig. 3.4). There were no significant interactions with year or treatment.

MICROBIAL PROCESSES

Mean microbial C and N were lower, although not significantly, in grazed plots compared to ungrazed plots (Table 3.2). Microbial C was positively correlated with soil C ($R^2 = 0.61$, $P \approx 0$; Fig. 3.5); plot 1 was not included in the analysis. Microbial C was negatively correlated with bulk density ($\tau = -0.44$, $P = 0.002$) and positively correlated with soil moisture ($\tau = 0.35$, $P = 0.02$) across treatments. The correlations were weaker for microbial N ($\tau = -0.39$, $P = 0.007$; $\tau = 0.28$, $P = 0.06$). Microbial N was correlated with resin N ($\tau = 0.51$, $P = 0.0004$) across treatments and had a weak yet significant relationship with net N mineralization ($\tau = 0.3$, $P = 0.04$).

Fatty acids

The cluster analysis separated the plots into 4 major groups (Fig. 3.6). Plot 1 is separated from all other plots in the dendrogram (Fig. 3.6). The grazed halves of plots 4 and 9 also have distinct EL-FAME composition. The remaining plots are divided into two large groups that do not distinguish between grazed and ungrazed. There is some incomplete separation between Horseshoe Park (plots 1-6) and Moraine Park (plots 7-12; Fig. 3.6).

Non-metric dimensional scaling did not generate a pattern associated with grazing treatments (Fig. 3.7). Environmental variables significantly correlated with the ordination axes were bulk density ($R^2 = 0.44$, $P = 0.003$), pH ($R^2 = 0.29$, $P = 0.03$), net N

mineralization ($R^2 = 0.30$, $P = 0.02$), soil moisture ($R^2 = 0.47$, $P = 0.008$), and microbial C ($R^2 = 0.48$, $P = 0.01$), the latter two being collinear. EL-FAME's were aligned largely on an axis of soil moisture; bacterial biomarkers were associated with the high soil moisture content of plot 1; bacterial and potential fungal biomarkers appeared closer to the intersection of the axes (Fig. 3.8). Biomarkers and plots were generally clustered near the center of the ordination plots. Moraine Park plots (7-12) were more tightly clustered than Horseshoe Park plots (1-6). The positioning of plots 2U and 3U could be associated with EL-FAME 21:0, which they share only with plot 1; this fatty acid is not a known biomarker for soil microbes.

EL-FAME's 22:0 and 24:0 were the only compounds to differ in relative mole percent between grazed and ungrazed treatments ($P = 0.02$, $P = 0.01$); these are not known biomarkers. The relative mole percent of bacterial EL-FAME's did not differ between treatments ($P > 0.05$) nor did the ratio of relative mole percent fungal EL-FAME's to relative mole percent bacterial EL-FAME's ($P > 0.05$); for this ratio we used 18:2 ω 6c as the fungal indicator. We did not find a significant correlation between the relative mole percent of 20:0, found in AMF and the relative mole percent of 16:1 ω 5c, an indicator of AMF mycelia (Olsson 1999) and did not include it as a fungal biomarker. The absence of 16:1 ω 9c from the sandier soils of plots 2, 3, and 4 (data not shown) suggests 16:1 ω 9c was a bacterial biomarker in this environment. The ratio of 17:0 cy to 16:1 ω 7c did not differ between grazed and ungrazed plots. The ratio of 19:0 cyclo to 18:1 ω 7c was not calculated due to the absence of 18:1 ω 7c in the samples. In sum, we did not find a relationship between soil microbial community composition and grazing.

Discussion

Grazing by *C. elaphus* in the riparian zones of Rocky Mountain National Park was associated with increases in soil bulk density, and decreases in soil moisture, soil C, soil N, and resin N. Studies of grazer effects on ecosystem processes often emphasize plant functional traits such as N content or the presence of defensive compounds (*e.g.* Ritchie *et al.* 1998). Declines in soil N have been attributed to replacement of high N forage species by low N forage species (Pastor *et al.* 1999, Harrison & Bardgett 2004). We could not attribute changes in soil N to herbivore-plant feedback mechanisms (Ritchie *et al.* 1998) because forage nutrient content was similar across species and functional groups (Schoenecker *et al.* 2004). *Salix* contain large quantities of defensive compounds but interaction with herbivory by ungulates, rather than insect herbivores, has not been documented (Committee on ungulate management in Yellowstone National Park 2002). In this study, plant functional traits seemed less important than the physical presence of grazing animals and the quantities consumed. The changes in soil C and N were likely the combined result of changes in physical soil traits and a decrease in the quantity of nutrient inputs.

The majority of research on soil compaction in natural systems has been driven by concerns about heavy equipment used during logging (Powers *et al.* 2005). In this context, soil compaction is generally perceived as positive if it benefits potential forest regrowth or negative if it reduces regeneration. The criteria for evaluating management options on lands set aside to preserve naturally functioning ecosystems differ from those employed on timber lands. The objectives are typically to preserve or restore natural ecological structure and function. Issues surrounding grazing in protected areas such as

national parks are therefore not about changes in soil productivity, as with the logging example, but about alterations to the system's naturally occurring state, as currently construed.

C. elaphus herds of Rocky Mountain National Park graze intensively in Horseshoe and Moraine Parks during winter and spring when soils are wet; sandy loam soils such as those at our study site are particularly susceptible to compaction when wet and slow to recover (Luckow & Guldin 2007). Grazing is associated with increasing bulk density (Drewry *et al.* 1999, Binkley *et al.* 2003) and decreasing soil moisture, porosity, infiltration, or hydraulic conductivity (Milchunas & Lauenroth 1993; Tracy & Frank 1998; Gill 2007; Drewry *et al.* 1999; Wheeler *et al.* 2002). The percentage difference in bulk density between the grazed and ungrazed sites in 2010 exceeded differences associated with tractor logging (Froehlich *et al.* 1985). Drier compacted soils slow flows of nutrients and water through the soil profile. Decreased flows can change microbially mediated processes such as nutrient cycling, storage, and immobilization.

The cessation of grazing in Horseshoe and Moraine Parks was associated with decreased soil bulk density and unchanged soil stocks of both C and N, suggesting a system rebounding from previous compaction. Grazing corresponded to a smaller decrease in bulk density, drier soils compared to ungrazed plots, decreasing stocks of both C and N, and decreased resin N. These effects were correlated with each other and with levels of microbial C and N. Microbial community composition was also aligned along axes of soil moisture and bulk density. If the current distribution of *C. elaphus* perpetuates an ecosystem with drier soils, higher bulk density, and decreasing supplies of C and N, a transition to a different ecological community with lower nutrient

requirements could occur, along with a change in microbial community composition. Faster nutrient cycling is not a likely alternative in cold wet soils. A question for future research is whether or not soil microbes are contributing to the observed decrease in soil C stocks by consuming stored soil C. Changes in the available of soil C substrates may also affect microbial biomass or community composition in the future, just as the previously documented decrease in soil N inputs (Schoenecker *et al.* 2004) is only now apparent in soil N stocks. Nitrogen limits plant growth at this site (Menezes *et al.* 2001); plant-microbe competition for N could increase if soil N continues to decline (Kaye & Hart 1997). We do not have sufficient data to identify the fate of the lost N. Grazing in *Populus tremuloides* (aspen) stands was associated with nitrate leaching at one site in an earlier study (Binkley *et al.* 2003), providing a potential mechanism in addition to the removal and translocation suggested by Schoenecker *et al.* (2004).

Exclosures create artificial environments when exclosed animals are an intrinsic part of the ecosystem. In this study, however, the exclosures were similar to a baseline because greater changes were seen in the soils of the grazed areas than of the ungrazed areas. We do not know what values for soil attributes, *Salix* growth, or *C. elaphus* populations might be within the range of natural variability in Rocky Mountain National Park. Undoubtedly, historical grazing caused some degree of soil compaction. Contemporary *C. elaphus* densities might affect soil processes differently if ecosystem structure were intact. Major predators of *C. elaphus* were probably *Ursus americanus* and *Ursus arctos* (black and grizzly bear; Zager & Beecham 2006), and *Canis lupus* (wolves; Eberhardt *et al.* 2003); the latter two were locally extirpated approximately a

century ago. Declining numbers of *C. canadensis* dams are also believed to influence current ecosystem characteristics (Singer & Zeigenfuss 2002).

The observed responses in Rocky Mountain National Park are generalizable to the interplay of nutrient inputs and soil dynamics rather than to grazing or riparian ecosystems. The importance of soil texture and moisture is reflected in the site-specific effects of grazing on soil C and N in New Zealand (Wardle *et al.* 2001), the divergent responses of African grasses to simulated grazing across a soil texture and moisture gradient (Anderson 1991), and soil organic matter dynamics in American grasslands (Parton *et al.* 1987). Milchunas & Lauenroth (1993) documented a global response of soil moisture to grazing but not a pattern in the response of soil C or N, possibly due to the role of physical soil characteristics. Parton *et al.* (1987) suggested that intensive grazing in grasslands would decrease soil organic matter, with nutrient inputs, soil texture and precipitation determining the rates at which this would occur. We do not have sufficient data to partition soil nutrient losses between changes in soil physical attributes and declining nutrient inputs. Our findings suggest that ecosystem responses to grazing in this montane riparian zone are more consistent with the nutrient input and soil texture-based model of Parton *et al.* (1987) than with models based on plant functional traits.

Park managers are faced with difficult choices when native animals are associated with ecosystem degradation in their original, now human-altered, habitat. Preserving soil resources and protecting nutrient cycles in the park's riparian zones requires redistribution or reduction of the ungulate population. Park initiatives have included construction of larger exclosures and, more recently, a culling program; hunting is prohibited in American national parks. Herd reduction can also be accomplished by

supplying national park elk to re-introduction programs in the eastern United States (e.g. Larkin et al. 2003). We suggest measures that affect soils and *C. elaphus* distribution simultaneously, such as raising water levels where *C. canadensis* has decreased. Higher water levels might slow the loss of soil C and redistribute *C. elaphus* on the landscape; the small dams placed in a subset of the study sites did not affect a large enough area to influence ungulate distribution. We also advise monitoring the condition of *Salix* in the increasingly dense exclosures and thinning if needed to maintain healthy source populations. Preferred policy in American national parks is to allow natural regulation of ecosystems. Where dynamics are significantly altered, intervention might be necessary to maintain ecosystem function.

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Supporting information

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

Table S1: Soil data from individual plots, 2010.

Table S2: Soil data from individual plots, 1998.

Table 3.1. Effect of location on soil variables (mean and coefficient of variation) in 1998 and 2010. *P*-values are from Student's *t*- test for normally distributed data and from the Mann-Whitney-Wilcoxon test for non-normal data.

	1998			2010		
	Horseshoe	Moraine	<i>P</i>	Horseshoe	Moraine	<i>P</i>
Soil moisture* (%)	23 (0.26)	38 (0.29)	0.0006	27 (0.07)	32 (0.11)	0.13
Bulk density (kg L ⁻¹)	0.89 (0.43)	0.69 (0.25)	0.02	0.66 (0.46)	0.62 (0.34)	0.38
Soil C (g kg ⁻¹)	82.7 (1.49)	68.7 (0.33)	0.04	65.87 (1.41)	59.76 (0.36)	0.03
Soil N (g kg ⁻¹)	6.16 (1.6)	4.96 (0.33)	0.04	4.26 (1.44)	4.28 (0.34)	0.02
Soil C (kg m ⁻²)	4.92 (0.46)	6.67 (0.21)	0.08	3.82 (0.39)	6.3 (0.12)	< 0.001
Soil N (kg m ⁻²)	0.34 (0.57)	0.48 (0.23)	0.07	0.25 (0.51)	0.47 (0.15)	< 0.001
Soil C:N	16.18 (0.34)	13.9 (0.08)	0.21	16.4 (0.18)	13.4 (0.4)	0.001
Resin N	3.6 (1.2)	6.9 (1.04)	0.09	6.2 (0.88)	6.5 (1.59)	0.02
Net min. N (g m ⁻²)	0.3 (1.76)	1.28 (1.04)	0.02	0.56 (1.1)	2.06 (0.43)	<0.001
Microbial C (g kg ⁻¹)				1.26 (1.7)	0.6 (0.2)	0.06
Microbial N (g kg ⁻¹)				0.13 (1.6)	0.1 (0.22)	0.07
Microbial C:N				9.75 (0.31)	7.6 (0.18)	0.01

*Excluding plot 1

Table 3.2. Treatment differences (mean and coefficient of variation) in soil variables for 1998 and 2010. *P*-values are from Student's *t*- test for normally distributed data and from the Mann-Whitney-Wilcoxon test for non-normal data.

	1998			2010		
	Ungrazed	Grazed	<i>P</i>	Ungrazed	Grazed	<i>P</i>
Soil moisture* (%)	34 (0.41)	28 (0.28)	0.05	32 (0.35)	27 (0.28)	0.06
Bulk density (kg L ⁻¹)	0.75 (0.42)	0.83 (0.38)	0.05	0.54 (0.43)	0.73 (0.34)	0.001
Soil C (g kg ⁻¹)	72.47 (1.16)	78.92 (1.18)	0.47	69.84 (0.91)	55.79 (1.27)	0.03
Soil N (g kg ⁻¹)	5.65 (1.41)	5.47 (1.11)	0.73	4.91 (0.93)	3.78 (1.14)	0.006
Soil N (kg m ⁻²)	0.37 (0.48)	0.45 (0.36)	0.03	0.35 (0.42)	0.37 (0.44)	0.63
Soil C (kg m ⁻²)	5.19 (0.37)	6.4 (0.32)	0.47	5 (0.34)	5.11 (0.35)	0.73
Soil C:N	16.18 (0.35)	14.47 (0.12)	0.21	15.13 (0.17)	14.71 (0.18)	0.23
Resin N	7.02 (1.09)	3.47 (0.94)	0.06	6.2 (0.88)	2.91 (1.59)	0.06
Net min. N (g m ⁻²)	1 (1.2)	0.66 (1.6)	0.15	1.45 (0.79)	1.17 (0.88)	0.34
Microbial C (mg g ⁻¹)				0.98 (1.72)	0.86 (1.59)	0.71
Microbial N (mg g ⁻¹)				0.12 (1.56)	0.09 (1.32)	0.63
Microbial C:N				8.38 (0.21)	8.98 (0.38)	0.13

*Excluding plot 1



Fig. 3.1. *Salix* (willow) in an enclosure in August, 2010. Grazed willows are in foreground. Fence is 2.4 m high.

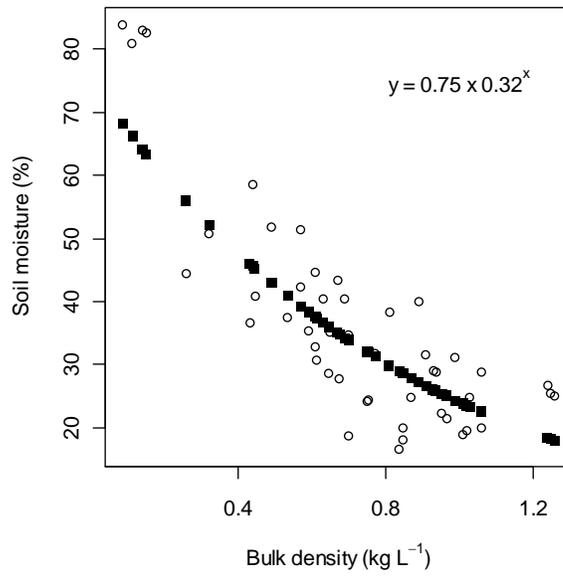


Fig. 3.2. Soil moisture, including both years and treatments, as a function of bulk density. Solid markers are fitted values. $R^2 = 0.66$, $P \approx 0$.

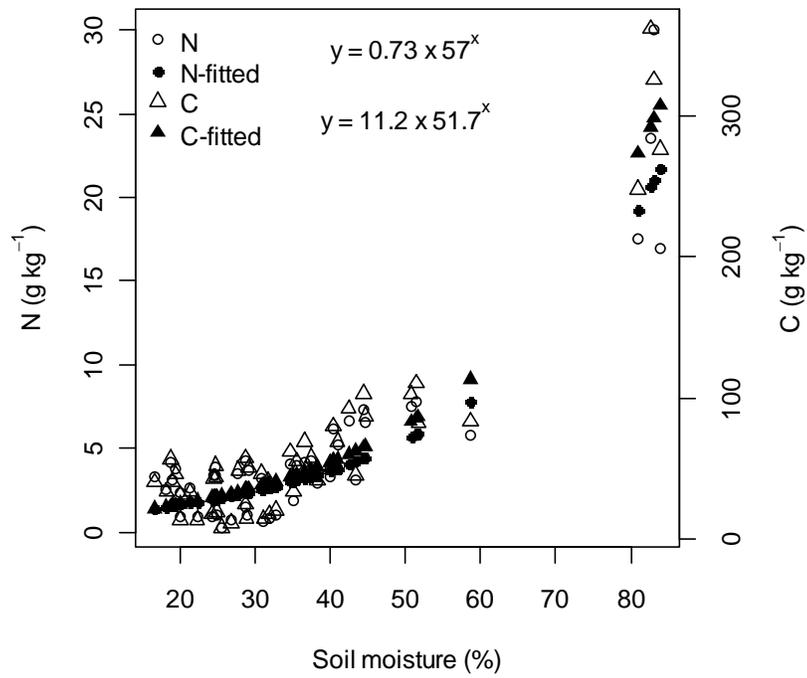


Fig. 3.3. Soil N and C (mass per mass dry soil) as a function of soil moisture. Data include both treatments and years. Nitrogen: $R^2 = 0.52$, $P \approx 0$. Carbon: $R^2 = 0.64$, $P \approx 0$.

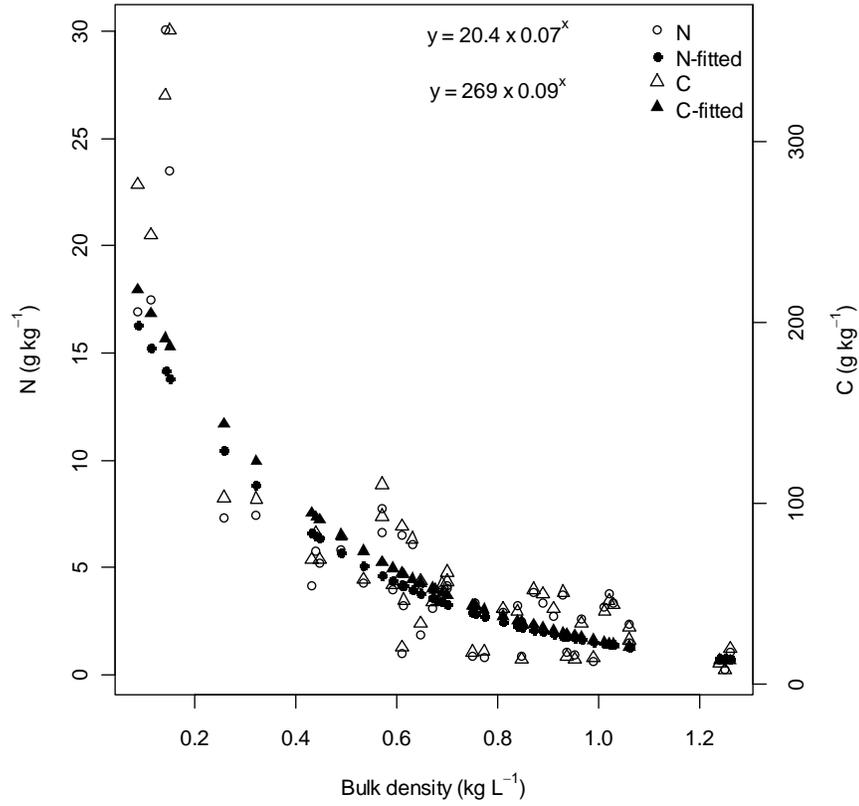


Fig. 3.4. Mass of soil N or C per mass dry soil as a function of soil bulk density including both treatments and years. Nitrogen: $R^2 = 0.62$, $P \approx 0$. Carbon: $R^2 = 0.7$, $P \approx 0$.

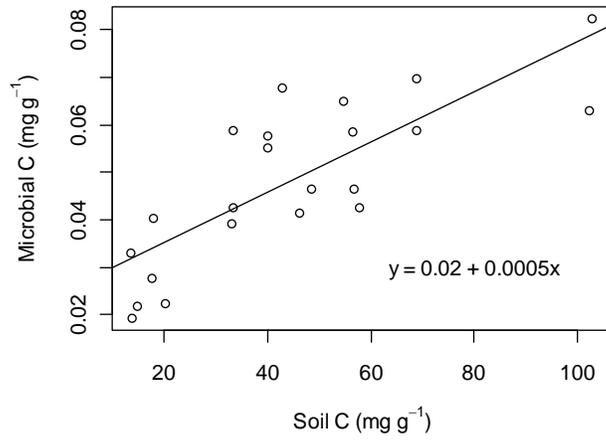


Fig. 3.5. Microbial C as a function of soil C. $R^2 = 0.61$. $P \approx 0$

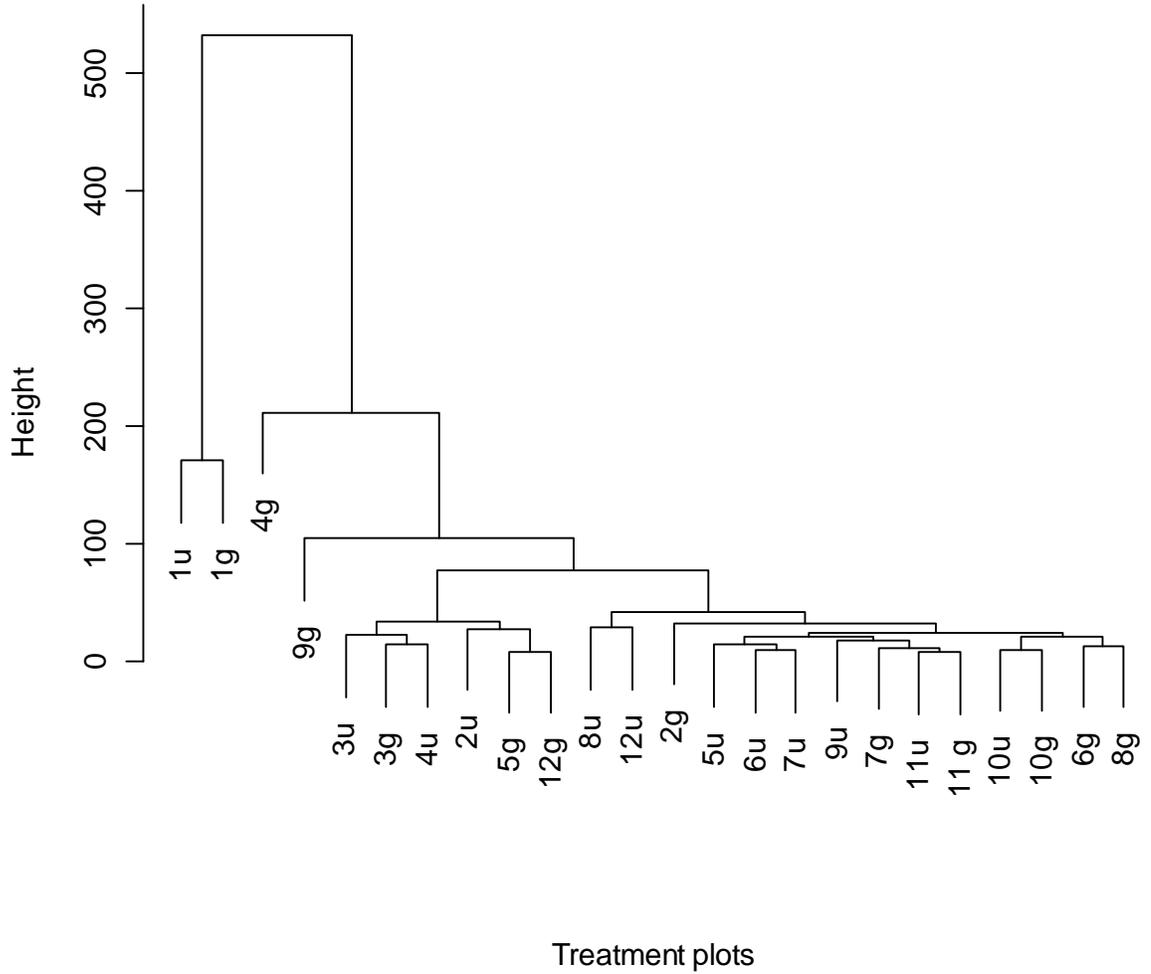


Fig. 3.6. Clustering of treatment plots according to the relative mole percentage of EL-FAME's identified with each plot. No treatment effect is apparent.

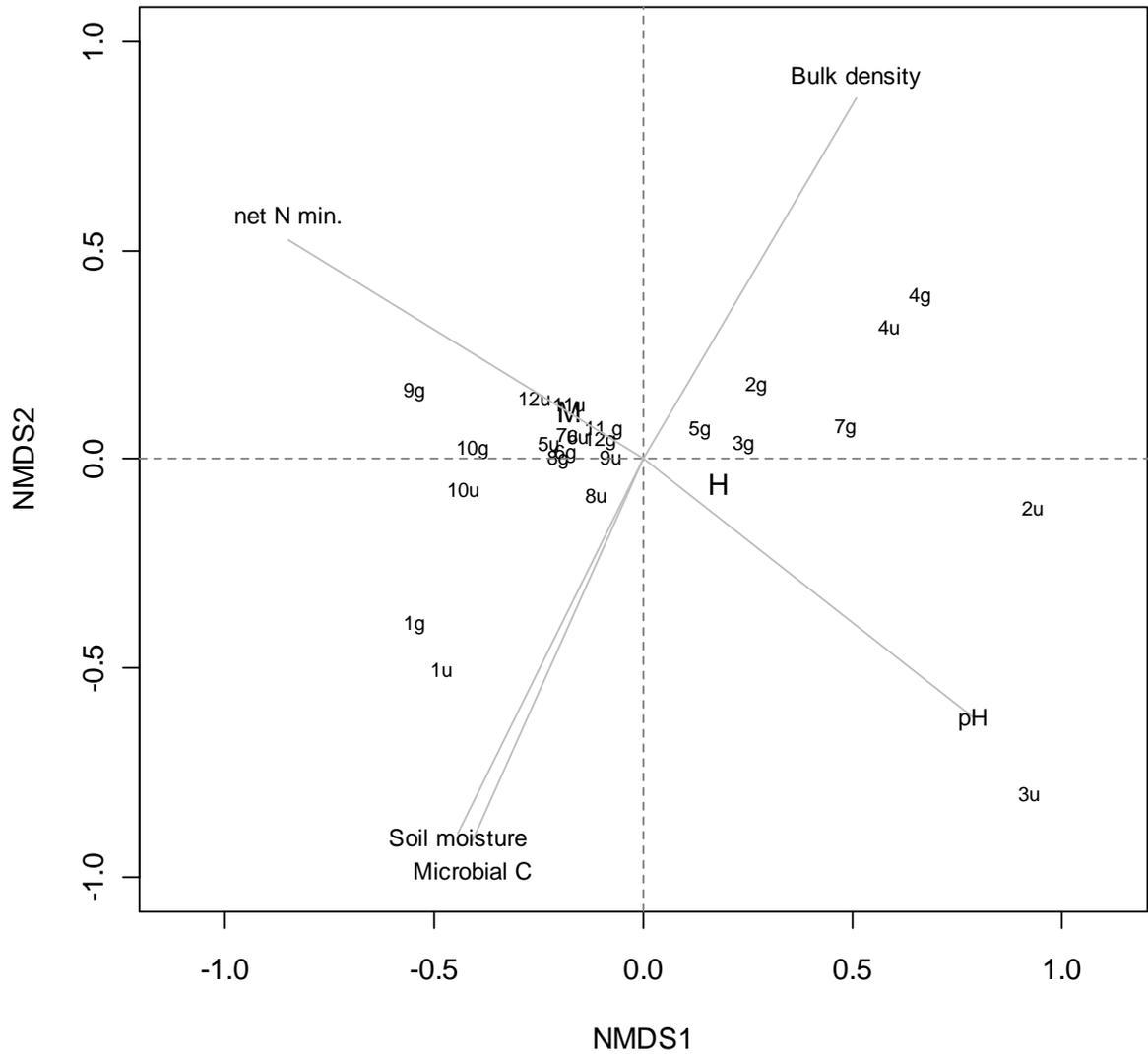


Fig. 3.7. Ordination of treatment plots according to microbial community composition, with an overlay of significant environmental variables. H indicates Horseshoe Park. M indicates Moraine Park. No treatment effect is apparent.

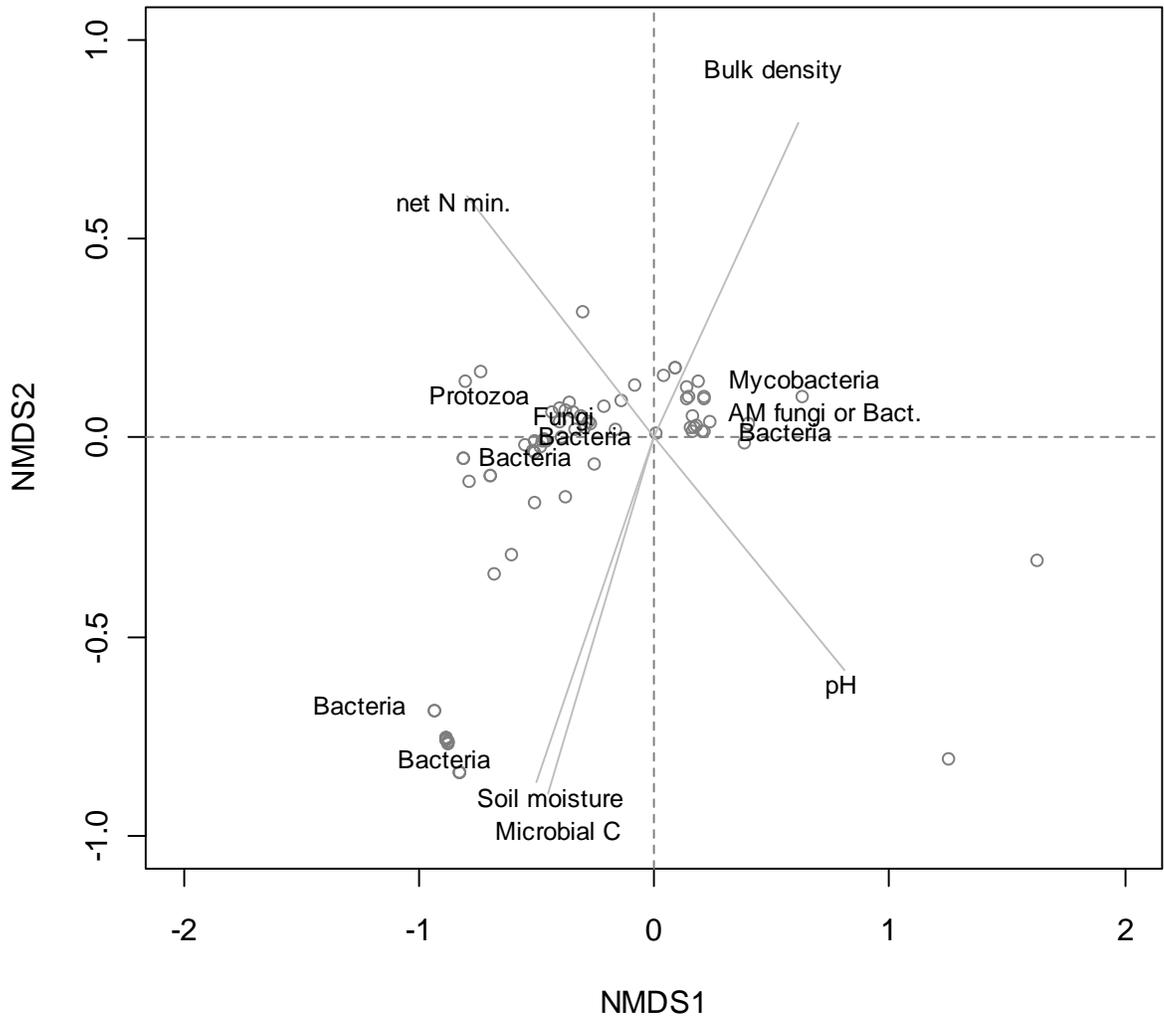


Fig. 3.8. Ordination of microbial community composition, as indicated by EL-FAME's, with an overlay of significant environmental variables. The most apparent pattern is the clustering of bacterial indicators with soil moisture and microbial C. This cluster is plot 1, as seen in Fig. 3.7.

Table S1. Soil characteristics by plot in 1998. Horseshoe and Moraine Parks are identified as H and M. Ungrazed and grazed treatments are identified as U and G.

Valley	Plot	Treatment	pH	Bulk density (kg L ⁻¹)	Soil moisture (%)	N (g kg ⁻¹)	C (g kg ⁻¹)	C:N	net N min. (g m ⁻²)	resin N	Microbial C:N	Microbial C (mg g ⁻¹)	Microbial N (mg g ⁻¹)
H	1	U	5.11	0.11	81	17.5	248.1	14.2	0.75	6.71	8.9	6.31	0.71
H	1	G	5.31	0.09	84	16.9	276.1	16.3	0.46	NA	11.1	5.17	0.47
H	2	U	5.46	0.61	33	1.0	20.3	20.8	0.13	0.41	18.0	0.22	0.01
H	2	G	4.9	0.77	32	0.8	17.8	21.3	0.21	2.51	9.9	0.40	0.04
H	3	U	5.2	0.75	24	0.9	17.5	19.4	0.17	0.55	8.7	0.28	0.03
H	3	G	5.57	0.94	29	1.0	14.9	14.4	0.57	2.59	8.8	0.22	0.02
H	4	U	5.07	0.85	20	0.9	13.5	15.6	0.74	1.15	8.3	0.33	0.04
H	4	G	5.11	0.95	22	0.9	13.7	14.5	-0.03	0.96	12.6	0.19	0.02
H	5	U	4.92	0.43	37	4.2	68.9	16.5	2.33	6.31	7.4	0.59	0.08
H	5	G	5.21	0.65	35	1.9	33.4	18.0	0.49	2.68	9.0	0.43	0.05
H	6	U	4.86	0.85	18	2.5	33.1	13.2	0.21	2.44	6.9	0.39	0.06
H	6	G	5.37	0.97	21	2.6	33.3	12.7	0.68	1.75	7.3	0.59	0.08
M	7	U	4.98	0.70	19	4.2	56.4	13.6	0.73	10.25	6.2	0.58	0.09
M	7	G	5.24	0.84	17	3.3	40.0	12.3	1.56	0.85	11.3	0.55	0.05
M	8	U	4.51	0.32	51	7.5	102.3	13.7	3.37	13.20	8.2	0.63	0.08
M	8	G	4.46	0.76	24	3.3	42.8	12.8	1.27	3.23	7.1	0.68	0.10
M	9	U	4.74	0.26	44	7.3	102.9	14.1	2.01	7.58	8.0	0.82	0.10
M	9	G	4.76	0.65	29	4.2	56.8	13.5	3.16	3.07	7.9	0.47	0.06
M	10	U	4.71	0.45	41	5.2	68.8	13.1	1.68	5.93	7.7	0.70	0.09
M	10	G	4.34	0.59	35	3.9	54.6	13.8	2.87	4.92	8.1	0.65	0.08
M	11	U	4.75	0.67	28	3.5	48.5	13.8	2.93	17.96	6.4	0.47	0.07
M	11	G	4.47	0.61	31	3.2	46.2	14.3	0.83	6.29	7.1	0.41	0.06
M	12	U	4.7	0.53	37	4.3	57.8	13.5	2.34	1.85	5.9	0.43	0.07
M	12	G	4.77	1.01	19	3.1	39.9	12.7	1.94	3.20	7.4	0.58	0.08

Table S2. Soil characteristics by plot in 1998. Horseshoe and Moraine Parks are identified as H and M. Ungrazed and grazed treatments are identified as U and G.

Valley	Plot	Treatment	pH	Bulk density (kg L ⁻¹)	Soil moisture (%)	N (g kg ⁻¹)	C (g kg ⁻¹)	C:N	net N min. (g m ⁻²)	resin N
H	1	U	5.11	0.14	83	30.1	325.7	10.8	1.90	14.18
H	1	G	5.31	0.15	83	23.5	361.6	15.4	1.56	9.76
H	2	U	5.46	0.99	31	0.6	14.1	22.1	0.26	0.29
H	2	G	4.90	1.24	27	0.7	11.1	15.7	0.05	0.19
H	3	U	5.20	1.25	26	0.2	7.7	32.2	-0.18	1.69
H	3	G	5.57	0.91	32	2.7	41.6	15.2	0.18	0.99
H	4	U	5.07	1.06	29	1.5	24.3	16.5	0.28	1.94
H	4	G	5.11	1.26	25	1.0	19.4	18.6	-0.23	0.93
H	5	U	4.92	0.70	35	4.0	61.7	15.3	0.38	5.79
H	5	G	5.21	0.93	29	3.7	50.6	13.6	0.07	1.05
H	6	U	4.86	1.06	20	2.4	31.3	13.2	0.32	1.25
H	6	G	5.37	1.03	25	3.4	43.5	12.9	-0.07	5.24
M	7	U	4.98	0.87	25	3.8	52.2	13.6	1.16	11.26
M	7	G	5.24	1.02	19	3.8	45.8	12.1	0.39	7.70
M	8	U	4.51	0.44	59	5.8	83.3	14.4	0.94	26.86
M	8	G	4.46	0.57	42	6.7	92.5	13.9	1.02	0.86
M	9	U	4.74	0.49	52	5.8	81.8	14.1	3.55	9.68
M	9	G	4.76	0.63	40	6.1	80.0	13.1	0.43	1.47
M	10	U	4.71	0.61	45	6.5	87.1	13.4	2.79	2.86
M	10	G	4.34	0.57	51	7.8	110.3	14.2	0.68	4.36
M	11	U	4.75	0.67	43	3.1	45.2	14.7	-0.44	6.63
M	11	G	4.47	0.81	38	2.9	41.3	14.0	0.19	7.06
M	12	U	4.70	0.69	40	4.0	55.2	13.9	0.99	1.82
M	12	G	4.77	0.89	40	3.3	49.4	14.8	3.65	2.01

CHAPTER 4

Carbon isotope responses to fertilization, density, and age in a wet tropical forest of

Eucalyptus saligna

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Submitted to Tree Physiology

Summary

We evaluated the sensitivity of stable carbon isotope discrimination (Δ) in stemwood cellulose of *Eucalyptus saligna* to fertilization, planting density, and age to determine whether treatment responses of gross primary production and canopy shading corresponded to changes in Δ . We compared the isotopic data to data on physiological and structural responses to the treatments at the same site. Fertilization increased photosynthetic capacity (A_{\max}) and gross primary production (GPP) but did not alter sap flux per unit leaf area or canopy conductance. Age and density treatments had little effect on conductance or photosynthetic capacity but were associated with differences in GPP and leaf area index. Stemwood Δ did not vary among treatments (mean = 19.08‰, se = 0.08). This result is consistent with the theory that an equilibrium between canopy conductance and canopy assimilation can maintain intercellular CO₂ concentrations (C_i) in changing environments. Adjustments in the ratio of sapwood area to leaf area helped maintain almost constant levels of water flux per unit leaf area as leaf area increased. Plentiful soil water and low atmospheric vapor pressure deficits may have prevented changes in the relative rates of assimilation and conductance, resulting, by inference, invariant C_i . We cannot discount the possibility that stemwood Δ muted or blurred treatment effects. Stemwood and foliar Δ are often poorly correlated and research confirming the relationship between stemwood Δ and canopy gas exchange is scant. We recommend additional development of validated methods for inferring canopy processes from stemwood Δ , particularly in the wet regions of the world.

Introduction

Plant tissues can be a natural archive, preserving information about physiological processes at the time the tissue was produced (Dawson *et al.* 2002). Analyses of plant material produced under contrasting natural conditions or experimental treatments provide information on how plant growth might respond to varying climate, changing atmospheric CO₂ levels, or direct human manipulation. Credible predictions of plant response to changing environments depend, in part, on skilled interpretation of this record.

The stable isotope ratios of carbon compounds (Δ) in plants are one natural archive of physiological function. Formation of the physiological record begins when CO₂ diffuses into the plant stomata, with simultaneous discrimination against ¹³C, a naturally occurring heavy isotope of carbon (C). The balance between the rate at which CO₂ diffuses through the substomatal region and the rate at which the CO₂ is used to create plant sugars is manifested in the intercellular carbon dioxide concentration (C_i) and thus in Δ . The relationship between C_i and Δ is described by Hubick et al. (1986) as

$$\Delta = a - d + (b - a) C_i/C_a \quad 1$$

where a is the diffusional discrimination at the stomatal pore (4.4‰), b is discrimination during the carboxylation reaction (26‰), C_i is the CO₂ concentration within the stomatal pore, and C_a is the atmospheric CO₂ concentration, as in the formulation of Farquhar et al. (1982). The variable d incorporates unmeasured parameters that influence net assimilation, including mesophyll conductance, the decrease in the concentration of CO₂ from the substomatal region to the chloroplast, and photorespiration (Hubick et al. 1986).

By rearranging equation 1, we can use Δ to solve for C_i . This estimate of C_i provides insight into the balance between diffusion and assimilation of CO_2 .

The stemwood of trees is one of the more accessible plant tissues and a natural candidate for isotope-based inference. Using Equation 1 as a foundation, Francey and Farquhar (1982) established the theoretical basis for inferring plant canopy processes from the carbon isotope ratios of stemwood. As with any proxy, we must be aware of conditions under which such inference is more or less successful. In the case of carbon isotopes, mechanisms internal to the plant can confound interpretation of Δ , as can canopy processes that vary without altering C_i .

Interpreting stable isotope ratios of plant tissues at increasing distance from the chloroplast is challenging. One of the greatest distances, both spatially and temporally, is from leaf to wood. Carbon compounds in wood have been repeatedly transported and transformed, possibly with concomitant fractionation (e.g., Damesin et al. 2003) or mixing (e.g., Keel et al. 2007). Additionally, these compounds likely originated from various parts of the canopy with different levels of light and nitrogen (N). Although the Δ of wood is considered a spatially and temporally integrated indicator of C_i , the process of integration and its effects on wood Δ are still being elucidated (e.g., Pate and Arthur 1998, Cernusak et al. 2003, Schulze et al. 2006, Kodama et al. 2008, Geßler et al. 2009). Nonetheless, stable carbon isotope ratios from wood have been consistent with changes in physiological processes in many temperate and semi-arid environments (e.g., Tans and Mook 1980, Leavitt and Long 1989, Martin and Sutherland 1990, Dupouey et al. 1993, McDowell et al. 2003)

Carbon stable isotope ratios might not be a strong indicator of plant responses to abiotic factors in well-watered environments (Hubick et al. 1986, Korol et al. 1999, Warren et al. 2001). Although the specific signals that prompt stomatal opening and closure are hotly debated, we know empirically that stomata are likely to be open during daylight hours if soil water availability is high, vapor pressure deficit (D) is low and light levels are high. Under these conditions, conductance is unlikely to limit the availability of CO_2 for assimilation (Farquhar and Sharkey 1982). Few studies of the relationships among environmental variables, gas exchange, and isotopic discrimination have occurred under conditions where water was essentially unlimited. As plant physiologists increasingly focus on wet tropical regions where soil and atmospheric water are plentiful, we must determine the applicability of methods originally developed in areas with seasonal water deficits.

We took advantage of an existing experiment to study the relationship between wood cellulose Δ and fertilizer and density treatments in a very wet environment. An experimental forest of *Eucalyptus saligna* provided a study site where the ratio of precipitation to transpiration was 10:1 and average daytime vapor pressure deficit (D) was less than 0.5 kPa (Hubbard and Ryan 2004). Results from this experiment showed that short-term fertilization temporarily doubled water-use efficiency (Hubbard and Ryan 2004). Long-term fertilization increased photosynthetic capacity and gross primary production (GPP) compared to controls but production decreased over time by 25% to 50%, depending on the treatment (Ryan et al. 2004). Trees grown at high density with continuous fertilization were shorter and had twice the leaf area index (LAI) compared to control trees grown at low density (Ryan et al. 2004). Fertilized trees had higher foliar

nitrogen concentrations than non-fertilized trees and assimilation rates increased with nitrogen content (Ryan et al. 2004). Our objective was to determine whether or not wood cellulose Δ would reflect the strong response of the trees to the treatments.

We hypothesized that the higher photosynthetic capacity of fertilized trees would be associated with decreased discrimination against ^{13}C (lower Δ) compared to controls. We predicted that stemwood from trees in the high-density treatments would indicate increased discrimination against ^{13}C compared to the low-density treatment, or decreased discrimination against ^{13}C if soil water became limiting. The hydraulic limitation hypothesis (Ryan and Yoder 1997) suggested a reduction in conductance and therefore increased discrimination against ^{13}C in the older, taller plots compared to the 1998 planting. Finally, we tested for a change in Δ over time that might mirror the change in GPP (Ryan et al. 2004); the cause of the long-term change in GPP has not been identified.

Materials and Methods

Study site

The study site is a 4.5 hectare experimental forest of *Eucalyptus saligna* on the windward side of the island of Hawaii (19°50'28.1"N, 155°7'28.3"W); the site elevation is 350m. Mean annual temperature is 21°C (Binkley et al. 1992), with slight seasonal variation. Maximum daily air temperature is usually between 24° and 28° C, peaking around day of the year 220. The mean annual precipitation of approximately 4000 mm is evenly distributed among the seasons (Debell et al. 1989), with an occasional drier month in spring or fall. The average daytime vapor pressure deficit (D) is 0.49 kPa (Hubbard et al. 2004). Maximum daily photosynthetically active radiation exceeds 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$

during all months of the year. Soils are deep (> 2m) thixotropic, isothermic Typic Hydrudands (Binkley et al. 1992). The site was planted in sugarcane for 80 years prior to the establishment of the experimental forest in 1994.

Trees were planted as seedlings in 1994 in a 2 x 3 factorial design of 18 plots in three randomized blocks; three more plots were planted in 1998 to study of the effects of age on physiology and production. Treatments in the 30 x 30 m plots included high density planting (10 000 ha⁻¹) and minimal fertilization; high density and high fertilization; low density planting (1111 ha⁻¹) and minimal fertilization; low density and high fertilization; low density and short-term fertilization; and low density, high fertilization, younger (1998 planting) trees. Control plots were fertilized at planting and at 6 months (as detailed in Hubbard et al. 2004). High fertilization plots were fertilized quarterly throughout the study period; short-term fertilizer plots received the same treatment as the control plots during the first year and the same quarterly treatment as the high fertilizer plots starting in April, 1998. Additional details and results of the treatments are in Binkley and Resh (1999), Giardina and Ryan (2002), Barnard and Ryan (2003), Binkley et al. (2004), Hubbard et al. (2004), and Ryan et al. (2004).

An additional site was used to extend the age sequence of the sample. Located within 400m of the main site, this 5 ha experimental forest was planted in *E. saligna* and *E. grandis* in 1983 (Schubert and Whitesell 1985). The spacing at planting was 2 x 2 m, with 70% mortality over the next 15 years (Giardina and White 2000). Fertilization was resumed in 1998 in one 30 x 30 m treatment plot and an adjacent unfertilized control area (Giardina and White 2000).

Treatment effects on Δ

We tested for significant differences in Δ between the control and continuous fertilizer treatments at both spacings for the years 1996 and 2000. To test for differences in Δ between the control and short-term fertilization treatments, we compared values from three trees per treatment in January 1998 (pre-treatment), June 1998, August 1998, February 1999, and May 1999. We then compared these results to the treatment effects on water use efficiency described in Hubbard et al. (2004). We tested the effect of age on discrimination by comparing Δ values from the same trees over time and from different aged trees at the same time. We compared these results to values of foliar Δ from trees of different ages (Barnard and Ryan 2003).

Biomass, flux, and physiological data

For the models and analysis in this study, we used previously published data collected at the same site. We use biomass, production and respiration data from Giardina and Ryan (2002), Giardina et al. (2002), Barnard and Ryan (2003), Hubbard et al. (2004), and Ryan et al. (2004). Gross primary production for each treatment was approximated as the sum of empirical estimates of foliage and wood net primary production, foliage and wood respiration, and total belowground carbon allocation (Ryan et al. 2004). Photosynthetic capacity was assessed by estimation of maximum assimilation rates (A_{\max}) and maximum carboxylation velocity (V_{cmax}) (Barnard and Ryan 2003, Ryan et al. 2004). Canopy conductance was estimated from individual tree sap-flux measurements scaled to the stand level (Hubbard et al. 2004).

Isotopic methods

We cored trees in 2004 to obtain wood samples representative of the range of tree ages and the fertilization and density treatments (Table 4.1). The eucalyptus trees at this site did not form annual rings because stemwood production occurs throughout the year. There was also no visible distinction between early- and late- wood. We used diameter measurements collected every 4 to 8 weeks throughout the study to estimate the diameter of each tree at different points over time. The location on a core that corresponded to each time period was calculated by converting diameter increments to radial growth after correcting for bark thickness. We used a Dremel™ tool with a 3/32" (2.38 mm) diameter diamond bit to remove fine-grain particles of wood from locations along the cores that coincided with the dates of interest. Cellulose was extracted from the wood using the method of Leavitt and Danzer (1993). The cellulose was analyzed for $\delta^{13}\text{C}$ on a Eurovector Elemental Analyzer coupled to a Micromass Isoprime isotope ratio mass spectrometer operated in continuous flow mode at Los Alamos National Laboratory's Stable Isotope Lab in Los Alamos, NM, USA. Nitrous oxide was removed by gas chromatography and corrections for ^{17}O (Craig 1957) were done for all runs. Overall precision for ^{13}C was 0.06‰ (n=41).

We used equation 2 to derive estimates of Δ from the wood cellulose $\delta^{13}\text{C}$. Δ allows us to make interannual comparisons of isotopic ratios despite changing atmospheric concentrations of ^{13}C .

$$\Delta = (\delta^{13}\text{C}_{atm} - \delta^{13}\text{C}) / (1 + (\delta^{13}\text{C}/1000)) \quad 2$$

where $\delta^{13}\text{C}$ and $\delta^{13}\text{C}_{atm}$ are the isotopic ratios of plant material and the atmosphere, respectively, relative to the VPDB standard (Farquhar et al. 1982). We used $\delta^{13}\text{C}_{atm}$ from the Mauna Loa observatory on the island of Hawaii (Keeling et al. 2005).

Given the continuous, rapid, and non-seasonal diameter growth of the trees at this site, we expected time lags between assimilation and wood production to be minimal. A constant offset between wood and foliage Δ has been found in many cases (e.g., Panek and Waring, Park and Epstein 1961, Leavitt and Long 1986, Schleser 1992, Damesin and LeLarge 2003). Δ of wood and cellulose may also differ by a constant offset (Leavitt and Long 1986, Marshall and Monserud 1996, Elhani et al. 2005). A constant offset would not obscure the treatment effects that are the object of this study. Werth and Kuzyakov (2006) suggested that Δ of specific carbon pools in plants is related to the percentage of assimilate allocated to those pools. In our study, the ratio of wood production to GPP varied among treatments (Ryan et al. 2004) so we tested for a statistically significant relationship between Δ and the ratio of wood production to GPP.

Statistical analysis

All comparisons of Δ involved repeated measures, either within trees or within plots. When the sample size was large enough, we used a linear mixed effects model (Pinheiro et al. 2009) to test for differences between treatments. When sample sizes were small, we used Welch's t-test for comparisons of two treatments, recognizing that t-tests do not account for repeated measures. For multiple comparisons, we used Tukey's HSD test. We used major axis regression (Legendre 2008) to estimate the correlation between Δ and the ratio of wood production to GPP in 1998, the year of the short-term fertilizer addition. The strength of the relationship between Δ and the ratio of wood production to

GPP over all years was tested with a linear mixed effects model. The significance level was $\alpha = 0.05$ for all tests. All statistical analyses were done in R 2.10.1 (R Core Development Team 2009).

Results

Wood cellulose Δ was nearly invariant over the 20 year age comparison and the 6 combinations of fertilization and spacing. The range of values for Δ was 18.2‰ to 21‰ with a mean of 19.8‰ (se = 0.08, n = 51). Following equation 1, the consistent value of Δ indicates C_i also differed little among treatments. The ratio C_i/C_a averaged 0.68 (se = 0.004; n = 41) for all measurements made on trees planted during the 1990's.

Effect of year and age on tree-ring Δ

At the plot level, mean (se) Δ for 1 to 2 year old trees in 1982 (n=3), 1996 (n=11), and 2000 (n=3) was 19.84‰ (0.53), 20.28‰ (0.14), and 19.32‰ (0.1), respectively ($P > 0.26$). We concluded there was no general time trend in Δ that might confound our analysis of an age trend. We then compared Δ within trees over time to test for a trend with age. Within a tree, discrimination estimated from the wood formed in 1982 and the wood formed in 2002 did not differ ($P = 0.4$). Plot-level mean (se) Δ of trees planted in 1994 was 20.05‰ (0.16) in 1996 and 19.85‰ (0.16) in 2000 ($P = 0.38$). The plot-level mean (se) Δ of trees planted in 1998 was 19.32‰ (0.1) in 2000 and 19.61‰ (0.07) in 2002 ($P = 0.08$). We tested for the relationship proposed by Werth and Kuzyakov (2006) and did not find a correlation between Δ and wood production either over all years ($P = 0.24$) or when short-term fertilizer was applied (CI on the slope = +24.5 to -11.2). The hypothesis that Δ would reflect the changes in productivity as trees aged was not supported.

Effect of fertilization and spacing on Δ

Average wood cellulose Δ did not differ significantly among combinations of spacing and control or continuous fertilizer in 1996 and 2000 (all $P > 0.77$); Figure 4.1). Tree-ring Δ also did not respond specifically to spacing ($p = 0.87$) or continuous fertilization ($P = 0.4$). The minimum average (se) Δ from any treatment plot was 19.86‰ (0.13); the maximum was 20.13‰ (0.31). The Δ of the short-term fertilizer plots and the control plots did not differ ($P = 0.49$, $df = 19$, Figure 4.2). The mean difference between treatments was 0.22‰. The hypothesis that Δ would respond to fertilization, spacing, shading associated with increased LAI, or increasing rates of assimilation was not supported (Figures 4.3 and 4.4). Increasing water use efficiency also did not affect Δ .

Foliar Δ

Foliar Δ from Barnard and Ryan (2003) differed significantly between 1 year old and 5 year old trees whether compared by canopy layers ($P = 0.016$) or bulked ($P = 0.03$). Mean (se) Δ of bulked leaves was 21.77‰ (0.31) for younger trees and 23.74‰ (0.46) for older trees.

Discussion

The constancy of stemwood Δ across treatments, years, and ages does not support our research hypotheses that differences in water use efficiency, stand density, fertilization, GPP, or tree age would be reflected in the carbon stable isotope ratio of stemwood cellulose. We consider several reasons why changes in canopy processes were not reflected in Δ . We first explore the possibility that changes in rates of assimilation occurred in tandem with changes in rates of conductance, yielding invariant Δ and C_i . We then consider the effect of high water availability on wood Δ . We discuss the

significance of discrepancies between foliar Δ and wood Δ and, finally, ask whether post-photosynthetic fractionation affected Δ .

Δ , C_i , and equilibrium in gas exchange

The lack of a nitrogen fertilization effect on Δ or gas-exchange is consistent with other studies that indicate transient or no effects of nitrogen fertilization at the leaf or canopy levels (e.g., Mitchell and Hinckley 1993, Korol et al 1999, Elhani et al. 2005, Balster et al. 2009, Brooks and Coulombe 2009). Over thirty years ago, Cowan (1977) proposed that changes in assimilation rates may be balanced by changes in transpiration rates. This would result in constant intercellular CO_2 concentrations and little or no change in Δ . With various modifications, the equilibrium theory has been supported at the leaf and plant levels in the ensuing years (Davis 1978, Wong et al. 1979, Wong et al. 1985a, Wong et al. 1985b, Wong et al. 1985c, Yoshie 1986) and is incorporated in standard models of gas exchange (e.g., Ball, Woodrow, and Berry 1987, Baldocchi 1994, Leuning et al. 1995). Increased rates of assimilation due to higher N levels are commonly balanced by increased rates of conductance, with no net change in Δ .

At the stand level, the equilibrium between assimilation and conductance might be achieved, in part, by changes in plant architecture (Addington et al. 2006, McDowell et al. 2006). At our site, water use increased with age, density, and fertilization (Barnard and Ryan 2003, Hubbard et al. 2004). In older trees, water supply was maintained by an increase in the ratio of sapwood area to leaf area and a decrease in minimum leaf water potential, compared to younger trees (Barnard and Ryan 2003); in fertilized trees, water supply was maintained by an increase in sap flux per unit sapwood area compared to control trees (Hubbard et al. 2004). These structural and physiological changes

facilitated the movement of large quantities of water to the photosynthetic sites of the leaves. Leaf-specific hydraulic conductivity decreased with tree age but this measurement was made at the whole-tree, rather than the branch, level (Barnard and Ryan 2003). Given that water flux per unit leaf area did not differ between younger and older trees (Barnard and Ryan 2003), branch leaf-specific hydraulic conductivity could also have been the same. Leaf-specific hydraulic conductivity of branches has been strongly correlated with maximum photosynthetic capacity in rainforest trees (Brodrigg 2000), with foliar $\delta^{13}\text{C}$ of *Pseudotsuga menziesii* (Douglas-fir) (Panek 1996), and with stomatal opening (Saliendra et al. 1995). The equilibrium between conductance and assimilation is modeled by Katul et al. (2003) where $V_{C_{\max}}$ is a function, in part, of soil water status, plant hydraulic conductivity, and D . As long as the equilibrium is maintained, C_i/C_a and Δ will be fairly constant in the presence of increasing or decreasing plant growth.

Δ in wet environments

Empirical data suggest that Δ becomes less informative or predictable in very wet environments (Farquhar et al. 1989), where conductance is unchanging or unlimited. Hubick et al. (1986) observed that the correlation between plant yield and Δ in dry conditions could not be duplicated under well-watered conditions. Reduced variability of Δ in wetter conditions has been noted in contrasting forest types (Walcroft et al. 1997, Pate and Arthur 1998), with fertilization affecting Δ only in drier conditions (Högberg et al. 1993, Korol et al. 1999). Walcroft et al. (1997) intensively studied two *Pinus radiata* trees growing in mesic and xeric environments, respectively. Seasonal variation in canopy-level C_i derived from stemwood $\delta^{13}\text{C}$ was considerably muted compared to

variation in leaf-level C_i estimated from a process model combining leaf-level gas exchange and soil water processes; the muting of the signal was most evident in the mesic environment. The non-responsiveness of Δ to water availability when the ratio of precipitation to evaporation or potential transpiration exceeds 1 (Korol et al. 1999, Warren et al. 2001) or when annual precipitation exceeds 2000 mm (Leffler and Enquist 2002) could indicate that maximal conductance is possible in wet environments (Clearwater and Meinzer 2000). At our site, annual precipitation exceeded 4000 mm and the ratio of precipitation to transpiration was 10:1.

The humid environment could also have contributed to a decoupling of canopy and leaf-level gas exchange. Our physiological data are averaged over a plot-level canopy; Δ integrates fluxes within an individual tree crown. Tree-level measurements would be incompatible with stand-level measurements if boundary layer effects (Barnard and Ryan 2003, Hubbard et al. 2004) were large. Conductance was not measured in the high-density stands where both fertilizer treatments and denser shorter trees could have affected canopy gas exchange.

Δ in wood versus foliage

Few data exist that document how stemwood Δ integrates canopy gas-exchange processes, how the integration is weighted with respect to contributions from different parts of the canopy, or which responses of canopy gas-exchange must be captured by proxies such as leaf or stemwood Δ at the stand level. At our study site, the treatments were associated with differences in foliar Δ for the one and five-year old trees but not with differences in stemwood Δ for the two and four-year old trees. We have found only two studies (Walcroft et al. 1997, Klein et al. 2005) that attempt to validate the theoretical

relationship (Francey and Farquhar 1982) between stemwood Δ and canopy or leaf-level gas exchange on annual time scales; both studies included wetter and drier treatments. Klein et al. (2005) studied two 10-tree plots of *Pinus halepensis*, one irrigated and one control. The relationship between both wood and needle $\delta^{13}\text{C}$ and assimilation-weighted instantaneous gas-exchange was fairly consistent; in one of 3 years, however, foliar $\delta^{13}\text{C}$ responded significantly to irrigation and wood $\delta^{13}\text{C}$ did not (Klein et al. 2005).

Another case of an apparent isotopic signal in foliage that was not recorded in stemwood is found in a study of tropical trees in Brazil (Martinelli et al. 1998). We assigned leaf and wood $\delta^{13}\text{C}$ values from the appendices of Martinelli et al. (1998) to 5m height classes, similar to the classes in Figure 1 of that publication. In the figure, there appear to be significant differences in leaf $\delta^{13}\text{C}$, but not wood $\delta^{13}\text{C}$, as height increases to 30m. We tested for significant differences among the 15-20, 20-25, and 25-30 m height classes. Differences in $\delta^{13}\text{C}$ values for foliage approached statistical significance but $\delta^{13}\text{C}$ values for wood did not ($P = 0.07$ for foliage; $P = 1$ for wood; Tukey HSD), suggesting a damping of the signal in stemwood. A global meta-analysis by McDowell et al. (*in review*) also documented a much weaker relationship between tree height and tree-ring Δ than between tree height and foliar Δ .

In one of the seminal works on uses of carbon isotope data from tree-rings, Leavitt and Long (1986) found no correlation between $\delta^{13}\text{C}$ of leaves and tree-rings from the same trees. The direction of seasonal trends differed between C_i derived from tree-ring $\delta^{13}\text{C}$ and C_i derived from foliar $\delta^{13}\text{C}$ for a tree in a mesic forest (Figure 6 in Walcroft et al. 1997); the correlation between the two estimates was not quantified. At a coarser spatial scale, $\delta^{13}\text{C}$ from leaves and tree-rings of Douglas-fir across a climatic gradient

were only moderately correlated ($r^2 = 0.42$) and foliar $\delta^{13}\text{C}$ was more closely coupled to environmental variables than was $\delta^{13}\text{C}$ from tree-rings (Panek and Waring 1997). In contrast, Keitel et al. (2003) found leaf $\delta^{13}\text{C}$ relatively uncoupled with environmental conditions compared to $\delta^{13}\text{C}$ of phloem sap. MacFarlane et al. (2004) found a correlation between $\delta^{13}\text{C}$ of leaves and tree-rings ($R^2 = 0.52$).

Information on how to interpret a pattern that is present in foliar Δ and simultaneously absent in stemwood Δ is lacking, particularly with regard to inferring equilibrium values of C_i . A muting or blurring of differences in stemwood Δ across years, ages or treatments could be a significant source of error for inferences about invariant C_i at the leaf or canopy level. Estimates of photosynthesis based on sap flow measurements may obscure or exaggerate patterns important to calculations of net assimilation, depending on where data are collected (Ewers et al. 2007). Data that explain why stemwood often shows such a muted pattern compared to foliage are not available. Conclusions about the relative rates of gas exchange might differ depending on whether they are based on foliage or stemwood Δ ; we do not yet know how to interpret such contradictory evidence.

With the exception of the two papers mentioned above (Walcroft et al. 1997, Klein et al 2005), there have been few attempts to define, model, and validate the presumed relationships among stemwood $\delta^{13}\text{C}$, canopy gas exchange, and leaf-level gas exchange. Data from this and other studies indicate we should be cautious using stemwood Δ for to infer canopy gas-exchange until the relationship between the two has been validated.

Post-photosynthetic processes

Post-photosynthetic fractionation or enrichment or discrimination during wood respiration could have affected stemwood Δ , leading to erroneous estimates of C_i . Evidence that post-photosynthetic fractionation affects stemwood Δ is mixed (e.g., Leavitt and Long 1982, Schleser 1992, Badeck et al. 2005). Fractionation and enrichment processes are usually accounted for by a percentage, as with a and b in Equation 1. We cannot think of a likely process by which post-photosynthetic fractionation or enrichment would erase dissimilarities, making stemwood Δ uniform across treatments; this would require the fractionation coefficient to differ with nutrient availability, tree age, and stand density. Discrimination during wood respiration could, in principle, cause uniformity in stemwood Δ if respiration were substrate-specific. Our Δ values were derived from cellulose rather than whole wood so we do not believe substrate-specific respiration is the cause of the invariant Δ . Constant Δ would occur if Δ were related to phloem sugar content and this content did not vary among treatments (Pate and Arthur 1998). Translocation of photosynthate, although not anticipated in trees that grow quickly and continuously, could also blur the isotopic signal as would mixing of carbon pools during transport (Keel et al. 2007).

Conclusion

Our study showed that stemwood Δ was insensitive to changes in leaf area, gross primary production, net primary production, and photosynthetic capacity associated with fertility and density treatments and forest aging. An overview of related publications indicates that the relationship between instantaneous c_i/c_a and Δ of plant tissues might be specific to species, to the choice of gas-exchange parameter, and to the temporal scale of the

measurements (Keitel et al. 2003, Schulze et al. 2006, Geßler et al. 2009, Cernusak 2009a, Cernusak 2009b). We advise development of validated empirical methods for inferring changes in canopy processes from stemwood Δ under a range of environmental conditions.

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Table 4.1: Sampling scheme for stemwood Δ

Planting year	1980	1994	1994	1994	1994	1998
Density		High	High	Low	Low	Low
Fertilizer	Control	Control	Continuous	Control	Short-term	Continuous
Trees sampled	9	12	9	13	4	15
Plots sampled	3	3	2	3	3	3

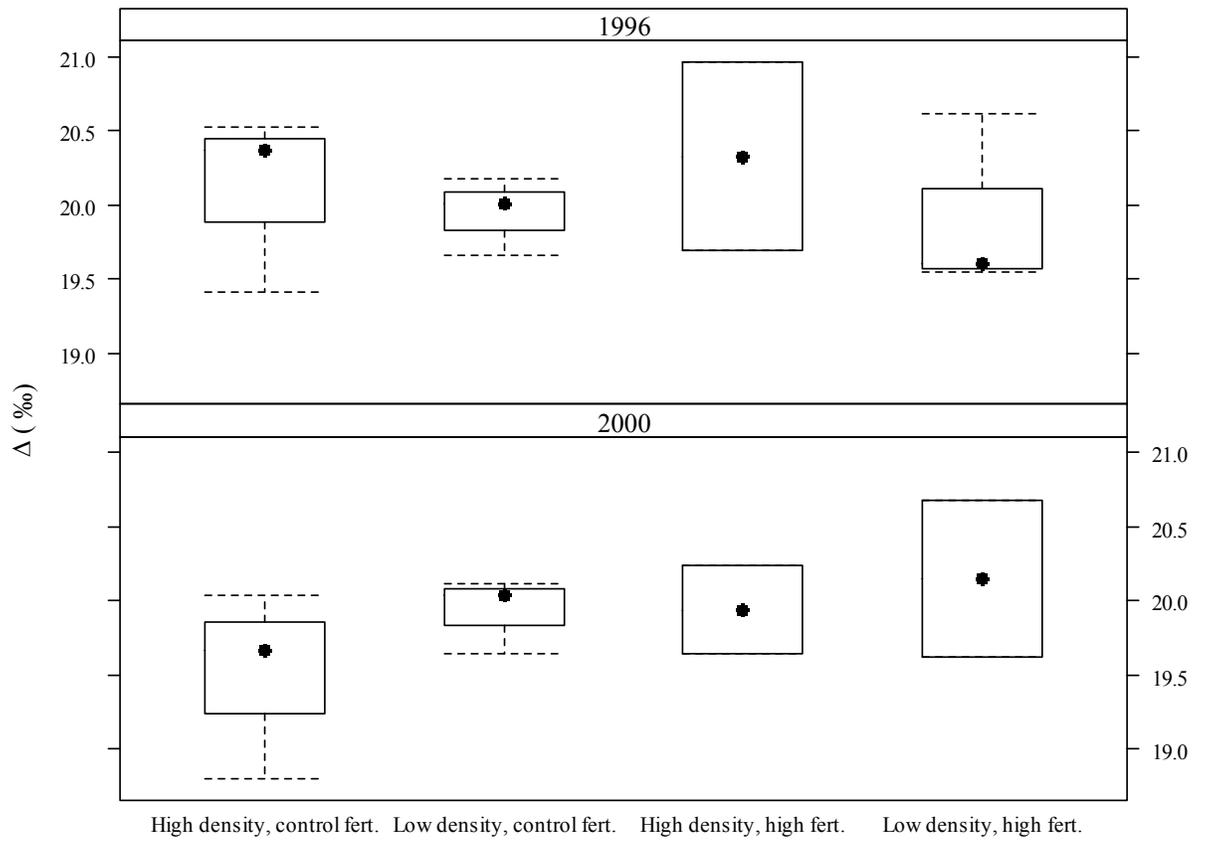


Figure 4.1. Δ does not differ among combinations of spacing and fertilizer.

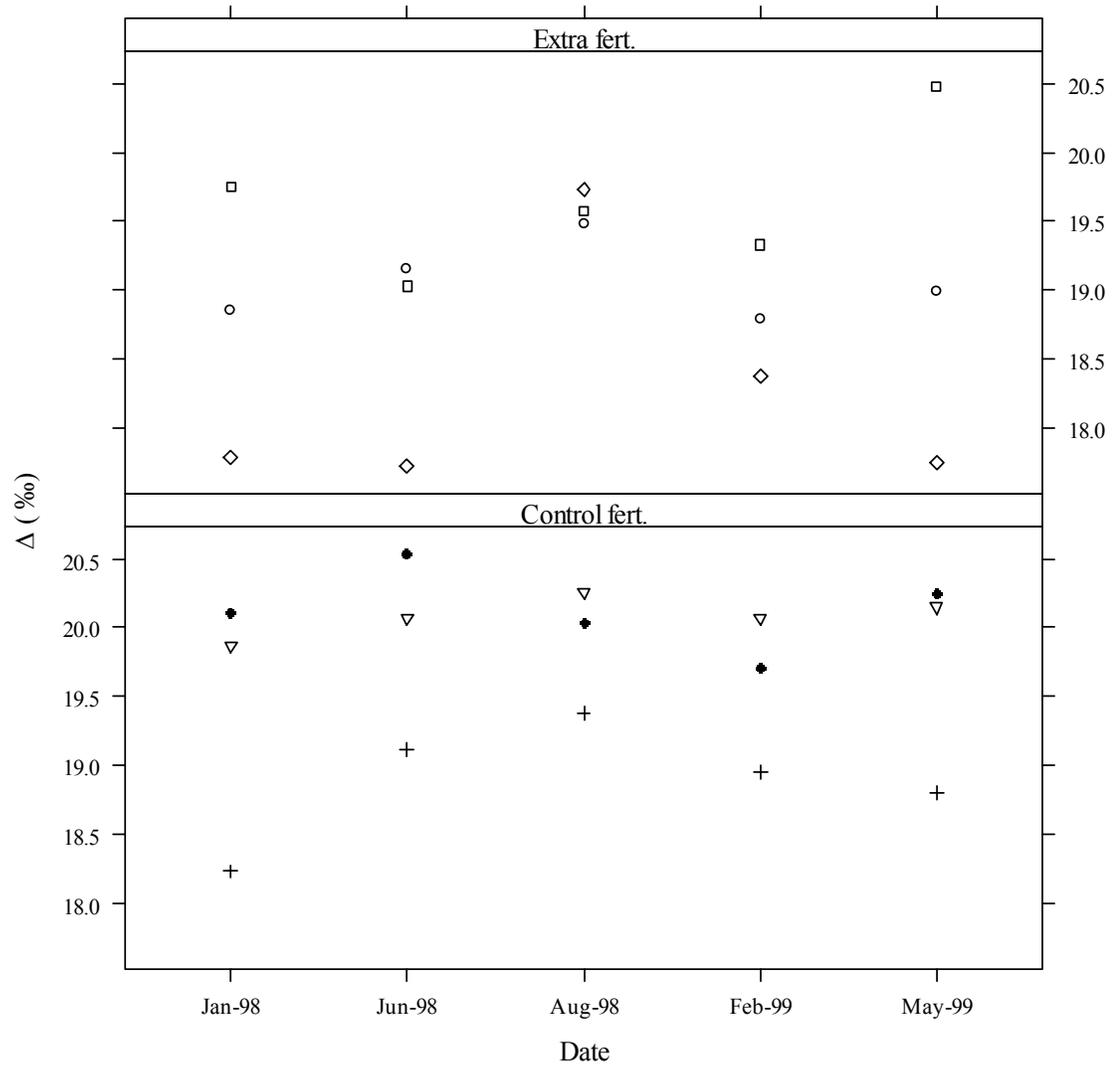


Figure 4.2. Δ does not respond to short-term fertilization. Fertilizer was applied in April, 1998. Symbols indicate individual trees. Spacing is low density.

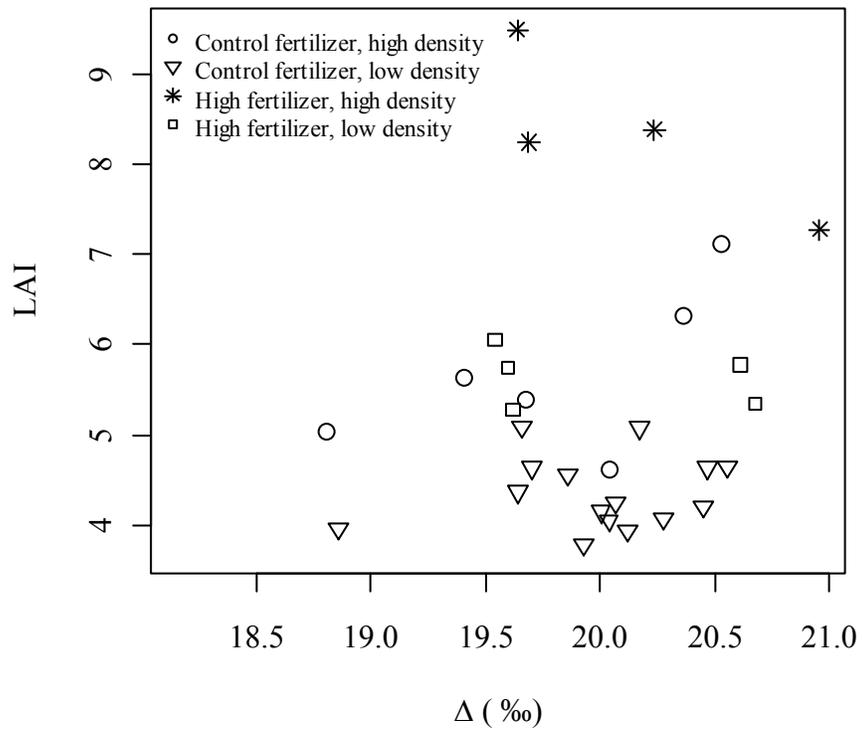


Figure 4.3. LAI varies with treatments; Δ does not.

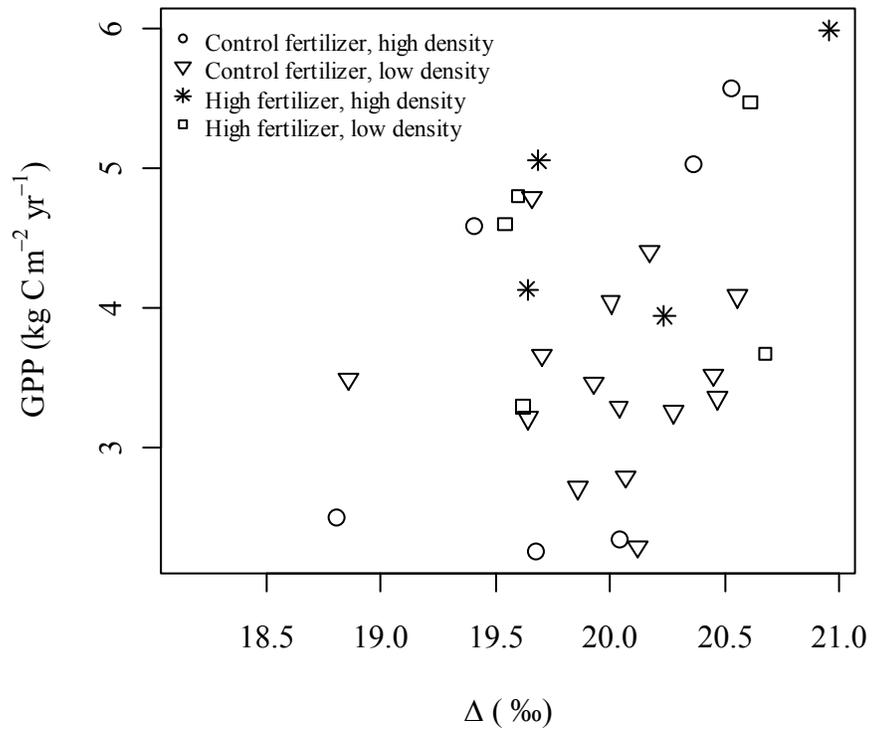


Figure 4.4. GPP varies with treatments; Δ does not.