EXOTIC GRASSES ALTER CONTROLS OVER SOIL NITROGEN DYNAMICS IN A HAWAIIAN WOODLAND

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Abstract. Exotic invasive grasses and fire have altered plant species composition in the seasonal submontane woodlands of Hawaii Volcanoes National Park. These changes have affected both structural and functional aspects of the plant community, which could have consequences for soil nitrogen (N) dynamics and N availability to plants. To determine if, when, and how soil N dynamics were altered by grass invasion, we measured net and gross N mineralization and nitrification during wet and dry seasons across three vegetation types: (1) experimental grass removal plots within unburned woodland created to simulate the native ecosystem that may have existed prior to invasion; (2) woodland invaded by grasses; and (3) invaded woodland converted to grassland by fire. Grass invasion into woodland shifted the timing, but not the amount, of N available. After conversion to grassland, N-cycling rates were 3.4 times greater. The wet season accounted for 35% of annual net N mineralization in the grass removal treatment, 75% in the grass-invaded woodland, and 90% in the grassland. Soil transplant experiments showed that this shift was caused by an interaction between season and the effects of vegetation type on controls over soil N transformations. The effects of grasses on soil organic matter (SOM) composition enhanced net N mineralization during the wet season, whereas their effects on microclimate depressed net mineralization during the dry season. During the wet season, higher net rates in the grassland were caused by higher rates of gross N mineralization, which were negatively correlated with SOM carbon : N ratio. During the dry season, lower net N mineralization rates in the presence of grasses were due to a larger proportion of gross mineralization being immobilized, which was positively related to soil moisture. These results indicate that changes in plant species composition can alter soil N availability through effects on microclimate as well as more frequently studied effects on SOM composition. Both of these effects appear to have consequences for ecosystem function and should be considered in the design of restoration strategies for ecosystems impacted by exotic plants.

Key words: biological invasion; ecosystem restoration; exotic grasses; fire; gross nitrogen; Hawaii; invasive species; mineralization; net nitrogen mineralization; soil nitrogen dynamics; woodland.

INTRODUCTION

Although human activities have led to clear and persistent changes in the composition of natural plant communities, we have only a limited understanding of the consequences of these changes for ecosystem function (Schulze and Mooney 1993, D’Antonio et al. 1999, Ehrenfeld and Scott 2001). Nitrogen (N) availability to primary producers is a key ecosystem function that appears to be particularly sensitive to the composition of the plant community. Plant species (Pastor and Post 1986, Wedin and Tilman 1990, Hobbie 1992, Reich et al. 1997), functional traits (Melillo et al. 1982, Berendse et al. 1987, Chapin 1993, Wardle and Nicholson 1996, Binkley and Giardina 1998, Burke et al. 1998), and diversity (Tilman et al. 1997, Hooper and Vitousek 1998) have been shown to affect N availability. In turn, alteration of N availability may drive further changes in composition (Tilman 1982, Chapin et al. 1986) and affect other ecosystem functions such as net primary productivity (Vitousek 1986).

The most often studied mechanisms by which plant species or functional groups of species affect N availability are via direct nutrient uptake (Tilman 1990, Tilman and Wedin 1991, Hooper and Vitousek 1998) and through effects on the rate of mineralization of N from soil organic matter, SOM (Wedin and Tilman 1990). In order to affect the latter, a plant species must alter a major control over soil N transformations. This includes alteration of the chemical composition of SOM, the microclimatic factors such as soil moisture and temperature that modulate rates of N mineralization, or both. Plant species and functional groups of species have been shown to have clear and predictable effects on SOM composition through the quantity (Hobbie 1992) and chemical composition of the litter that they produce (Pastor and Post 1986, Wedin and Pastor 1993).

The effects of plant composition on microclimate have received less attention, yet there is evidence that these types of effects may also be important for soil N
mineralization (Matson and Boone 1984, Wedin and Pastor 1993). Traits such as canopy architecture, rooting depth, and litter deposition have been shown to have effects on soil thermal (Van Cleve et al. 1983, Burke 1989, Das et al. 1995, Seastedt and Adams 2001) and hydraulic environments (Richards and Caldwell 1987, Dawson 1993, Schlesinger and Pilmanns 1998) and whole-stand microclimate (Bonan et al. 1995, Chapin et al. 1995, Baldocchi and Vogel 1996, Ripley and Archibold 1999). Several studies have linked the effects of a particular plant species or functional type on microclimate to soil N transformations in ecosystems where moisture (Burke 1989, Hibbard 1995) or temperature (Van Cleve et al. 1981, Seastedt and Adams 2001) are limiting to decomposer activity, but no studies have experimentally separated plant species effects on microclimate from effects on SOM composition and compared the relative importance of these two mechanisms.

In this study, our goal was to determine whether the changes in plant community composition associated with invasion by exotic grasses altered soil N availability in a seasonally dry woodland on the island of Hawai`i. Because of the marked seasonality of precipitation in this system, we inspected both annual and seasonal N fluxes to determine whether exotic grasses altered the timing of N mineralization. We addressed this by measuring annual and seasonal net N mineralization and nitrification across three vegetation types: (1) experimental grass removal plots within unburned woodland, created to simulate the native ecosystem that may have existed prior to invasion; (2) woodland invaded by grasses; and (3) invaded woodland converted to grassland by fire. We then examined the mechanisms through which vegetation composition affected soil N transformations, and how these mechanisms differed during wet and dry seasons.

First, we compared microclimate and SOM composition across vegetation types and across seasons. Second, we examined the relative importance of these controls over net N mineralization with reciprocal soil transplant experiments during the wet and dry season. Finally, because net rates are based on competing gross cycling processes (Hart et al. 1994a), we examined gross mineralization, immobilization, and nitrification to further understand the mechanisms underpinning the effects of vegetation type on net rates of mineralization.

**METHODS**

**Study area**

We conducted this study within the seasonal submontane woodland zone of Hawai`i Volcanoes National Park (HVNP), Hawai`i, near Kipuka Nene campsite (19°6’ N, 155°33’ W, elevation 850–900 m). Soils are young (< 500 yr) shallow (mean depth = 40 cm) entisols developed on ash deposits overlaying ~1000 yr-old pahoehoe lava (Wolf and Morris 1996). Precipitation averages 1500 mm/yr and is highly seasonal, with ~10% of annual precipitation falling between April and August (HVNP, Resource Management Division, Data Archives). Mean annual air temperature (T) is 20°C. The climate, soils, vegetation structure, and N pools in these woodlands have been described in detail elsewhere (Hughes et al. 1991, D’Antonio et al. 1998, Freifelder et al. 1998, Mack et al. 2001).

Historically, these were open-canopy woodlands of the native tree *Metrosideros polymorpha* (Myrtaceae), with an understory of native shrubs. Grasses were rare in these evergreen woodlands until the 1960s, when exotic *C.* grasses invaded following an expansion of grazing land outside of the park boundary (Smith 1985). Today there are no submontane woodlands in HVNP without exotic grasses in the understory (D’Antonio et al. 1998). *Schizachyrium condensatum* (Poaceae), a perennial bunchgrass native to Central and South America, is the most abundant grass in the understory. *Melinis minutiflora* (Poaceae), a stoloniferous perennial grass native to Africa, and *Andropogon virginicus* (Poaceae), a perennial bunch grass native to North America, are also present at low densities. Botanical nomenclature follows Wagner et al. (1990). Because the only grasses in all of our plots were exotic in origin, where we refer to “grasses” hereafter, we are referring solely to exotic ones.

Since grasses invaded in the 1960s, these woodlands have experienced a threefold increase in fire frequency and a 35-fold increase in number of hectares burned per fire (D’Antonio et al. 2000). The initial effects of fire on N cycling are short lived; increased rates of soil N mineralization and nitrification return to ambient levels within six months (C. M. D’Antonio, unpublished data), and < 2% of total ecosystem N is lost due to biomass burning (Mack et al. 2001). Consequently, the most lasting effect of fire is a change in vegetation composition: the woodlands are converted from a functionally diverse assemblage of shrubs, graminoids, and trees to exotic grassland with a few highly dispersed shrubs (Hughes and Vitousek 1993).

Primary production in nearby seasonal submontane forests (Vitousek et al. 1995) and the grass-dominated ecosystems that follow fire (C. M. D’Antonio, unpublished data) is N limited. Thus, changes in plant community composition that affect rates of N availability may ultimately have important consequences for primary production in this ecosystem.

**Vegetation types**

Our study sites consisted of three spatially interspersed vegetation types: (1) unburned woodland with grasses present (hereafter, Woodland + Grass or W+G); (2) a grass removal treatment in the unburned woodland (hereafter, Woodland or W), which simulated conditions that might have existed in the woodland prior to grass invasion; and (3) woodland burned both in 1970 and 1987, which converted to grassland (hereafter,
Grassland or G). This study design enabled us to examine the effects of native species, the interactive effects of natives and grasses, and the effects of grasses alone on soil N transformations.

In 1991, Woodland + Grass and Woodland treatments were randomly assigned to eight 20 × 20 m plots that had been selected to have similar native and exotic vegetation cover, soil depth, and aspect (D’Antonio et al. 1998). Standing aboveground biomass and necromass of exotic grasses were removed from the Woodland plots by clipping grasses just below the root crown. Roots were left intact to minimize soil disturbance. New grass seedlings were weeded from plots biannually between 1991 and 1997. We randomly selected four 20 × 20 m Grassland plots from burned woodland in 1993 with the same criteria used to select plots in the unburned woodland, except that their locations were constrained to areas contiguous to the unburned woodland that burned in 1970 and 1989. Pre-fire aerial photographs and ground surveys show that all plots in this study were at one time part of a continuous forest belt dominated by an open canopy of *Metrosideros polymorpha* (HVNP, Resource Management Division, Data Archives).

In 1994, vegetation in the Woodland vegetation type consisted of 100% native woody species cover and biomass. In the Woodland + Grass, grasses contributed 70% of the understory cover and 30% of understory biomass. In the Grassland, *Melinis minutiflora* made up 90% of the total cover and 70% of the total biomass (Mack et al. 2001). *Dodonaea viscosa* is the only native shrub that recruits into these grasslands following fire. It made up <10% of biomass in the Grassland plots from 1992 to 1998 (D’Antonio et al. 2000).

*Grass contributions to soil C pools*

Our primary goal with this experiment was to determine whether the rapidly cycling SOM pools in our vegetation types reflected current vegetation composition, and not historical composition (e.g., grass C inputs prior to grass removal treatment). We used the differences in the 13C:12C isotope ratio between SOM formed from C3 native litter vs. C4 exotic grass litter to determine the relative contributions of each group to total and labile SOM across vegetation types. We sampled leaves in January 1994 to determine the C isotope ratio of litter inputs from native species and grasses. We collected 10 leaves each from five individuals of dominant species in each replicate plot of each site, and dried the leaves in the lab at 70°C for 48 h. We sampled soils in January 1994 by taking four randomly selected 3.5 cm diameter × 15 cm deep cores from each replicate plot. Cores were pooled by plot and returned to the lab, where they were sieved to 2 mm and fine roots were removed by hand. For analysis of total C, subsamples were dried at 70°C for 48 h and then ground with a mortar and pestle. Additional subsamples were also dried at 100°C for determination of moisture content. For labile C, 10 g oven-dry equivalent subsamples of field-moist soil were weighed into cups, brought up to field capacity with deionized water, and placed into one 1-L mason jars fitted with stopcocks and incubated at 26°C. After 10 d, 10 mL of gas was removed from the jar headspace with a syringe, the entire volume of which was injected into a vacuum (Townsend et al. 1995). Plant tissue, soil, and gas were returned to University of California—Berkeley for isotopic analysis. We analyzed C isotope composition of CO2, plant tissue, and bulk soil on a Europa Scientific Tracer mass spectrometer (Europa Scientific, Crewe, UK). Stable carbon isotope ratio was calculated using delta (δ) notation with Pee-Dee belamite as the standard (Lajtha and Michener 1994). Site differences in the δ13C of leaves or soil pools were tested with a t test or one-way ANOVA, respectively. If necessary, data were ln(x + 1)-transformed to eliminate heterogeneity of variances. In cases where differences were detected, post hoc comparisons were carried out using the Bonferroni method. All statistical analyses were performed using Systat 7.0 (SYSTAT 1997). This protocol was used for all ANOVA analyses described hereafter, unless otherwise noted. The percentage of grass C in pools was estimated on a per plot basis using a simple mixing model constrained to the plot average C3 and plot average C4 leaf isotopic composition.

*Net rates of N mineralization and nitrification*

We used a series of intact soil core incubation experiments to determine the effect of changes in plant species composition on seasonal and annual net N mineralization, our proxy for plant available N. We measured net rates of NH4+ and NO3- production across the three vegetation types in situ incubations of intact soil cores capped with ion exchange resins (DiStefano and Gholz 1986, Hart and Firestone 1989). Nitrogen transformation rates were measured three times during the dry season (July 1994, and April and July 1995) and two times during the wet season (November 1994 and January 1995). Sampling was conducted within randomly selected subplots in each of the four replicate plots of each vegetation type. Within each subplot (two per plot in November, January, and April, four per plot on other dates), two 12 cm lengths of 3.4 cm diameter × 2 mm thick polyvinyl chloride pipe were pounded into the soil to 10 cm depth after the litter layer was removed. Cores were then withdrawn from the soil and one was immediately placed on ice and returned to the lab for determination of initial inorganic N concentration, gravimetric soil moisture, total percentages of C and N, and bulk density. The second core was capped on either end by nylon bags of 3-g dry mass mixed-bed ion exchange resins (J. T. Baker, Phillipsburg, New Jersey), and incubated in its original hole for 28 d.

Soil temperature at 5 cm depth in the bulk soil of each subplot was measured on four days during each incubation between 1100 and 1300 hours. Soil tem-
perature was also measured at 5 cm depth within the cores at the first time point. Temperature at 5 cm depth in cores was similar to bulk soil temperature in the incubation plot (paired \( t_{12} = 1.25, P = 0.24 \)). After incubation, cores were placed on ice and returned to the lab for determination of final mineral N pool sizes, soil moisture, and percentages of C and N.

In the lab, all soil cores were passed through a 2-mm sieve before extraction. For mineral N pool sizes, 15 g field moist soil was added to 75 mL of 2-mol/L potassium chloride (KCl), shaken for 1 min, and allowed to stand overnight. Solutions were then filtered through Whatman #1 filter paper pre-leached with 50 mL deionized water and 25 mL 2-mol/L KCl. Field-moist resins were agitated in 50 mL 2-mol/L KCl for 1 min and allowed to stand overnight. Filtered samples were frozen and shipped to University of California-Berkeley for analysis, where NH\(_4^+\) and NO\(_3^-\) concentrations were determined colorimetrically using a Lachat flow-injection autoanalyzer (Lachat, Milwaukee, Wisconsin). Soil subsamples for percentages of C and N were dried at 70°C for 48 h. Soils were ground to a fine powder with mortar and pestle, and analyzed at UC–Berkeley for percentages of C and N on a Carlo Erba NA 1500 Nitrogen Analyzer (Fisons Instruments, Saddlebrook, New Jersey, USA). Gravimetric moisture content was determined on samples dried at 100°C for 48 h. In January and July 1995, we also incubated a composited sample of initial cores in the lab as an index of potentially mineralizable C. We brought 10 g of field-moist soil up to water-holding capacity, placed it in a 1-L mason jar, and incubated it at 26°C. After 7 d, headspace gas was analyzed for [CO\(_2\)] on a Shimadzu mini-8 gas chromatograph (Shimadzu, Columbia, Maryland, USA) fitted with a methanizer and flame ionization detector.

Net rates of N mineralization and nitrification were calculated from the changes in μg N-(NH\(_4^+\) + NO\(_3^-\)) and N-NO\(_3^-\) per gram dry soil, respectively, over the incubation. Initial pool sizes were calculated for the <2 mm soil fraction as micrograms of N per gram of soil in the initial core. Final pool sizes were calculated as the sum of mineral N in the core and on the lower resin bag, divided by the mass of soil in the core. Annual net N and NO\(_3^-\) production per square meter was calculated on a seasonal and an annual basis for a 1 × 1 × 0.10 m\(^3\) cube of soil with a bulk density of 0.64 (Mack et al. 2001). We tested for the effects of vegetation type on soil moisture, soil temperature, percentages of C and N, C : N and mineralizable C at each time point, and on annual net N and NO\(_3^-\) production, using one-way ANOVA as previously described.

**Reciprocal soil core transplant experiment**

In January and July 1995, we used a reciprocal soil transplant experiment to further examine the mechanisms through which changes in vegetation composition affected net rates of N mineralization and nitrification. Exotic grasses differ strongly from native trees and shrubs in litter quality (Mack et al. 2001) and canopy structure (D’Antonio and Vitousek 1992), factors that we hypothesized would have impacts on SOM quality and soil microclimate. We used the incubation core method described previously, and removed two additional cores from each subplot, transplanting them to the other two vegetation types for incubation. Vegetation type of origin, then, was a test of the effects of SOM composition (hereafter, origin treatment) on transformation rates, and vegetation type of incubation was a test of the effects of microclimate (hereafter, incubation treatment). We used two-way nested ANOVA to test for main effects with incubation plot nested within incubation treatment, but orthogonal to origin treatment (Underwood 1997).

**Gross rates of N mineralization and nitrification**

Measures of net nitrogen mineralization and nitrification confound two or more concurrent gross N cycling processes: the production and consumption of NH\(_4^+\) and NO\(_3^-\) (Hart et al. 1994a). In order to further explore the mechanisms through which vegetation composition affected net N mineralization, we used a \(^{15}\)N isotope dilution method (Hart et al. 1994b) to estimate gross rates of mineralization, immobilization, and nitrification in each of the vegetation types. At the time of the reciprocal core transplant (January and June 1995), we collected three randomly selected 3.5 × 10 cm deep soil cores from each plot. Cores within a plot were bulked in January and analyzed separately in July. Cores were placed in 4-L polyethylene bags, where soil was mixed, rocks and plant parts >5 mm were removed, and soil subsamples were taken for determination of percentages of C and N and gravimetric soil water content as described earlier. The soil in the bag was then divided into two bags by mass; one was labeled with 99% \(^{15}\)N as \((^{15}\text{NH}_4)^{+}\)SO\(_4^−\), the other with 99% \(^{15}\)N as K\(^{15}\)NO\(_3^-\) (Cambridge Isotope Services, Boston, Massachusetts, USA). Our goal was to label soils with ~1 μg \(^{15}\text{NH}_4^+\) or 0.5 μg \(^{15}\text{NO}_3^-\)/g dry soil. The actual dilution resulted in the addition of 12.0 ± 1.1% (mean ± 1 se) of the total NH\(_4^+\) pool. Ambient soil NO\(_3^-\) concentrations were lower, resulting in the addition of 41.1 ± 4.5% of the total NO\(_3^-\) pool. Fifteen minutes after \(^{15}\)N addition, soil was subsampled from bags and immediately extracted in 2-mol/L KCl as described previously. Bags were then placed into the soil and retrieved after 24 h, at which time they were subsampled and extracted as we have described to determine final pool dilution.

We measured NH\(_4^+\) and NO\(_3^-\) extractable in KCl as previously described, and extracts were prepared for \(^{15}\)N isotopic analysis using a Teflon tape diffusion method (Sorensen and Jensen 1991, Stark and Hart 1998). Isotopic enrichment was determined on the Tracer Mass Spectrometer fitted with an automated CN analyzer de-
TABLE 1. Mean (and 1 SE) δ¹³C and percentage of C derived from grasses in soil organic C (SOC) pools in three vegetation types in a Hawaiian seasonal submontane woodland.

<table>
<thead>
<tr>
<th>Vegetation type</th>
<th>Total SOC</th>
<th>Labile SOC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ¹³C</td>
<td>Grass C (%)</td>
</tr>
<tr>
<td>Woodland</td>
<td>-25.5±(0.7)</td>
<td>4.1 (3.2)</td>
</tr>
<tr>
<td>Woodland + Grass</td>
<td>-25.2±(0.5)</td>
<td>4.3 (2.5)</td>
</tr>
<tr>
<td>Grassland</td>
<td>-22.5±(0.5)</td>
<td>31.1 (6.0)</td>
</tr>
</tbody>
</table>

Note: Means within a column with different superscript letters are significantly different (P < 0.05).

RESULTS

Grass contributions to soil C pools

Rapidly cycling soil organic C strongly reflected current vegetation type, confirming the utility of our experimental design for determining the effects of plant community composition on SOM composition. The carbon isotope ratio of labile C was lightest in the Woodland vegetation type and indicated that residual grass C no longer had an impact on this pool (Table 1). Delta values were significantly heavier in the Woodland + Grass and Grassland, where one-quarter and two-thirds, respectively, of labile C was derived from grasses (Table 1). Grass C made up a relatively small portion of the total soil organic C pool in both Woodland and Woodland + Grass. By contrast, one-third of the total soil C in Grassland was derived from grass C (Table 1).

Net mineralization and nitrification

Annual net N mineralization was substantially higher in the Grassland (13.6 ± 2.6 g N·m⁻²·yr⁻¹, mean ± 1 SE; P < 0.05) than in the Woodland (3.9 ± 1.3 g N·m⁻²·yr⁻¹) or Woodland + Grass (4.1 ± 1.1 g N·m⁻²·yr⁻¹; F₂,9 = 9.82, P = 0.005), primarily due to higher mineralization in the Grassland during the wet season (Fig. 1; F₂,9 = 13.89, P = 0.002). Mineralization was higher in the Woodland than in the Woodland + Grass (P = 0.05) or Grassland (P = 0.02) during the dry season (F₂,9 = 6.28, P = 0.02), but because rates were substantially lower in the dry season than in the wet season (Fig. 1), the greater mineralization in the Woodland at that time did not equal the much higher rate observed in the Grassland in the wet season. Annual net nitrate production did not differ among vegetation types or seasons (Fig. 1).
Soil microclimate and SOM composition across vegetation types

Vegetation type strongly affected soil microclimate on most sampling dates (Fig. 2, Table 2). Soil temperatures were lower and tended to be moister in vegetation types with grasses than in the Woodland at all time points except for November 1994 (Fig. 2). Across seasons and treatments, all soils were warmer and drier in April–July, and cooler and moister in November–January (Fig. 2). Although there were seasonal trends in temperature (5 cm depth) in Woodland (W), Woodland + Grass (W+G), and Grassland (G) vegetation types in a Hawaiian woodland. Asterisks indicate significant differences among sites ($P < 0.05$). See Table 2 for statistics. In the wet season, there was no evidence for differences in percentages of C or N, although the C:N ratio was significantly lower in the Woodland than in Woodland + Grass (Table 3). Mineralizable C was significantly lower in the Grassland than in the Woodland + Grass or Woodland (Table 3). In the dry season, soil percentage of N was higher and percentage of C tended to be lower in the Woodland than in either vegetation type with grasses, resulting in a significantly lower C:N ratio in the Woodland (Table 3).

<table>
<thead>
<tr>
<th>Date</th>
<th>Moisture (g/g)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
</tr>
<tr>
<td>Jul 1994</td>
<td>2, 9</td>
<td>48.60</td>
</tr>
<tr>
<td>Nov 1994</td>
<td>2, 9</td>
<td>1.14</td>
</tr>
<tr>
<td>Jan 1995</td>
<td>2, 9</td>
<td>2.9</td>
</tr>
<tr>
<td>Apr 1995</td>
<td>2, 9</td>
<td>5.10</td>
</tr>
<tr>
<td>Jul 1995</td>
<td>2, 9</td>
<td>14.24</td>
</tr>
</tbody>
</table>

**Notes:** November and January F values are for one-way ANOVA with df = 2, 9. All other F values are from one-way nested ANOVA, with df = 2, 9 for vegetation type and df = 9, 12 for plot nested within vegetation type. Nested statistics are not shown.

**Table 2.** One-way ANOVA results for the effect of vegetation type on soil moisture (0–10 cm depth) and temperature (5 cm depth) during in situ soil core incubations in 1994–1995 in Hawaii Volcanoes National Park; see Fig. 2 for means.

**Table 3.** Mean (and 1 se) and ANOVA statistics for soil microclimate and SOM composition variables during the measurement of net and gross rates in three vegetation types (W, Woodland; W+G, Woodland + Grass; and G, Grassland) in a Hawaiian seasonal submontane woodland.

**Notes:** Superscript letters within a season and row denote significant differences ($P \leq 0.05$). Wet-season F values are for one-way ANOVA with df = 2, 9. Dry-season F values are from one-way nested ANOVA, with df = 2, 9 for treatment and df = 9, 12 for plot by vegetation type (nested statistics are not shown).
TABLE 4. Two-way nested ANOVA results for wet-season (January 1995) and dry-season (July 1995) soil transplant experiments, which tested the effect of incubation (I) and origin (O) vegetation type on net nitrogen mineralization and net nitrification (μg N · [g soil]⁻¹·d⁻¹).

<table>
<thead>
<tr>
<th>Dependent variable and source</th>
<th>Wet season</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
</tr>
<tr>
<td>Net mineralization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubation†</td>
<td>2</td>
<td>0.30</td>
</tr>
<tr>
<td>Origin‡</td>
<td>4</td>
<td>7.85</td>
</tr>
<tr>
<td>I × O</td>
<td>18</td>
<td>0.25</td>
</tr>
<tr>
<td>Iplot(I)</td>
<td>9</td>
<td>3.62</td>
</tr>
<tr>
<td>O × Iplot(I)</td>
<td>18</td>
<td>2.83</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>107</td>
</tr>
<tr>
<td>Net nitrification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubation</td>
<td>2</td>
<td>0.37</td>
</tr>
<tr>
<td>Origin§</td>
<td>2</td>
<td>10.50</td>
</tr>
<tr>
<td>I × O</td>
<td>4</td>
<td>5.57</td>
</tr>
<tr>
<td>Iplot(I)</td>
<td>9</td>
<td>9.30</td>
</tr>
<tr>
<td>O × Iplot(I)</td>
<td>18</td>
<td>5.09</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>107</td>
</tr>
</tbody>
</table>

† Degrees of freedom (9) and MSE (0.0003) for Iplot(I) were used to test for differences among Incubation means.
‡ In post hoc tests for differences among Origin means, mean squares and degrees of freedom were pooled from I × O and O × Iplot(I) terms. Pooled df = 22, and MSE = 0.07.
§ O × Iplot(I) MSE (0.23) and df (18) were used to test for differences among Origin means.
¶ O × Iplot(I) MSE (0.23) and df (18) were used to test for differences among Origin means.

Relative effects of soil microclimate and SOM composition on net N mineralization

Soil organic matter composition controlled net rates of mineralization and nitrification during the wet season (January 1995). In the soil core transplant experiment, the origin treatment (SOM composition) had a strong effect on net mineralization, whereas incubation treatment (microclimate) had no effect (Table 4, Fig. 3). Cores originating in the Grassland mineralized more N than did all others cores, no matter where they were incubated. Net nitrification was similarly affected by origin, although origin interacted with incubation (Table 4). Cores originating in the Grassland had significantly higher nitrification rates than did cores originating in the Woodland, except in the Woodland + Grassland incubation treatment (Table 4, Fig. 3).

In the dry season (July 1995), microclimate controlled net mineralization. Cores incubated in the Woodland + Grassland and Grassland had lower net N mineralization rates than did cores incubated in the Woodland, regardless of origin (Table 4, Fig. 4). Neither microclimate nor SOM origin influenced net nitrification (Table 4). Bulk soils in the Woodland were significantly warmer and drier than soils in the Woodland + Grass or Grassland (Fig. 2). Core soil moisture, however, reflected both incubation and origin treatments, suggesting that vegetation composition altered both microclimate and moisture-holding capacity of SOM.
TABLE 5. One-way and nested one-way ANOVA statistics for gross rates of N transformations (dependent variables, measured as μg N·[g dry soil]⁻¹·d⁻¹) in three vegetation types (independent variables) in seasonal submontane Hawaiian woodland during wet (January 1995) and dry (July 1995) seasons.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wet season</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
</tr>
<tr>
<td>NH₄⁺ production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veg. type</td>
<td>2, 9</td>
<td>36.62</td>
</tr>
<tr>
<td>Plot(v.t.)</td>
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Notes: Wet-season F values are for one-way ANOVA with df = 2, 9. Dry-season F values are from one-way nested ANOVA, with df = 2, 9 for type and df = 9, 12 for plot nested within vegetation type, plot(v.t.).

Gross rates of N transformation

During the wet season (January 1995), the gross NH₄⁺ production rate differed significantly among all vegetation types (Table 5, Fig. 5), and was highest in the Grassland and lowest in the Woodland + Grass (Fig. 5). Gross NH₄⁺ consumption, however, did not differ between the Grassland and the Woodland, and was significantly lower in the Woodland + Grass (Fig. 5). As a result, the ratio of NH₄⁺ consumed:produced was significantly lower in the Grassland (0.74 ± 0.10, P < 0.05) than in the Woodland (1.14 ± 0.11), with Woodland + Grass intermediate (1.00 ± 0.07; F₂,9 = 4.51, P = 0.05), which may explain why there were higher rates of net mineralization in the Grassland in the in situ core incubations during this season (Fig. 1).

Gross mineralization was negatively correlated with SOM C:N (r₁₂ = −0.61, P = 0.03), but not to percentages of C and N, moisture, or temperature (data not shown). None of these covariates, however, altered the effect of vegetation type on the gross mineralization rate when included in ANCOVA. Other unmeasured aspects of SOM composition, such as the relatively lability of C and N compounds, may have been driving differences among vegetation types. The SOM C:N ratio alone appeared to explain differences in consumption because its inclusion in ANCOVA removed the effect of vegetation type on consumption (vegetation type, F₂,9 = 3.46, P = 0.09; C:N ratio, F₁,7 = 0.75, P = 0.42). The C:N ratio was negatively correlated with consumption across all treatments (r₁₂ = −0.67, P = 0.02).

During the dry season (July 1995), gross NH₄⁺ production did not differ among vegetation types (Table 5). NH₄⁺ consumption, however, was higher in the Grassland than in the Woodland or Woodland + Grass (Fig. 5), and a significantly larger proportion of N mineralized was consumed in the Grassland (1.3 ± 0.08 consumption:production, mean ± 1 SE) and Woodland + Grass (1.1 ± 0.12) than in Woodland (0.80 ± 0.17, vegetation type, F₂,9 = 4.04, P = 0.05; the nested term was not significant). Thus, lower dry-season in situ rates of net mineralization in vegetation types with

![Fig. 5. Mean (± 1 SE) gross NH₄⁺ transformations during the wet season (January 1995) or dry season (July 1995) in three vegetation types in a Hawaiian woodland: W, Woodland; W+G, Woodland + Grass; and G, Grassland. Bars within a season with different letters are significantly different (P < 0.05). See Table 5 for statistics.](image-url)
grasses than in Woodland (Fig. 1) may be explained by higher rates of consumption relative to production. SOM composition (percentages of C and N and C:N ratio) was not significantly correlated with gross NH$_4^+$ production or consumption during the dry season. By contrast, soil moisture was positively correlated with consumption ($r_{24} = 0.68$, $P = 0.01$), whereas temperature was negatively correlated ($r_{24} = -0.42$, $P = 0.05$). Moisture tended to be highest in the Grassland (Table 3) and appeared to be driving differences in consumption: its inclusion as a covariate removed the significant vegetation type effect (ANCOVA, vegetation type, $F_{2,9} = 2.43$, $P = 0.13$; moisture, $F_{1,9} = 4.43$, $P = 0.06$; nested terms are not shown). By contrast, inclusion of temperature had no effect on significance values (data not shown).

Gross NO$_3^-$ production rates were extremely variable within treatments and were not different among treatments at either time point (Table 5, Fig. 5). Gross nitrate consumption was affected by vegetation type during the wet season, but the significance of differences between means was marginal (Grassland > Woodland at $P < 0.08$, and Grassland > Woodland + Grassland at $P < 0.13$). There were no differences in consumption during the dry season. Rates were not correlated with microenvironment or SOM composition during either season. The ratio of NO$_3^-$ consumption:production was >1 for all vegetation types during both seasons, indicating that our $^{15}$NO$_3^-$ addition stimulated NO$_3^-$ uptake by microorganisms (Davidson et al. 1991) and may have obscured measurement of the gross processes driving differences in net nitrate immobilization during the wet season.

**FIG. 6.** Mean (±1 se) gross NO$_3^-$ transformations during the wet season (January 1995) or dry season (July 1995) in three vegetation types in a Hawaiian woodland: W, Woodland; W+G, Woodland + Grass; and G, Grassland. Bars within a season with different letters are significantly different ($P < 0.05$). See Table 5 for statistics.

### DISCUSSION

In this seasonal submontane woodland, changes in plant community composition associated with grass invasion appear to have altered the timing and amount of N available for plant uptake. Removal of grasses from the unburned woodland caused a 60% increase in the amount of N mineralized during the dry season, but no change in total annual mineralization (Fig. 1). Extrapolation of this finding to the initial invasion suggests that grasses have altered the percentage of annual net mineralization occurring during the dry season, from 65% before invasion to 25% after invasion. These effects on the timing of N mineralization alone may have several potential consequences at the ecosystem level. First, increased immobilization of nitrate in the dry season (Fig. 1) could lead to lower N losses through trace gas emissions or leaching losses (Likens et al. 1970, Vitousek and Matson 1984). Second, a greater proportion of annual mineralization occurring in the wet season, when plants are most actively taking up N (Mack 1998), may result in greater synchrony between N availability and plant growth.

Conversion of woodland to grassland via fire appears to have greatly increased plant available N in this system, a pattern observed in other systems with infrequent fires (Carreira et al. 1994, Ojima et al. 1994, Singh 1994, Kaye and Hart 1998). Net mineralization was 3.4 times greater in the grassland than in the unburned woodland. Greater than 90% of this mineralization occurred in the wet season, when SOM composition was the dominant control over rates of transformations. There is no evidence, however, that this increased N availability feeds back to increase primary productivity in the grassland. Although grass production is similar in the grassland and unburned woodland, total plant production and N uptake is lower in the grassland due to the loss of natives, and thus total plant N uptake only accounts for one-third of net mineralization, resulting in a decoupling between N availability and plant N (Mack et al. 2001). This decoupling is partially the result of an offset between the timing of grass growth and soil N mineralization (C. M. D’Antonio and M. C. Mack, unpublished manuscript).

**Controls over N mineralization**

Our data support the idea that plant functional types can alter N mineralization through substantial effects...
on microclimate as well as effects on SOM composition, the factor that has been more widely studied. We found that the relative importance of these two controls over net N mineralization depended on season and plant community composition. In our reciprocal transplant experiments, microclimate was the only significant control over net N mineralization during the dry season, whereas SOM composition was the only significant control over net mineralization during the wet season.

Support for seasonal dependence of controls over N mineralization has been found in many other studies (e.g., Schimel 1986, Richards et al. 1988, Burke 1989, Boone 1992, Fransluebbers et al. 1994). The results of our study and others suggest that the effects of plant composition on SOM quality will be most important for N mineralization when abiotic conditions are ameliorated, whereas microclimatic effects become most important when abiotic factors are limiting to microbial processes. Clearly, the effects of plant composition on N dynamics via microclimate should be strongest in ecosystems where abiotic conditions limit N transformations.

Our assessment of gross N transformations during the dry season showed that lower net mineralization in vegetation types with grasses was probably due to the positive effect of moisture on gross NH$_4^+$ immobilization relative to production. Although production did not differ among the three vegetation types, the proportion of production that was immobilized was higher in the two vegetation types with grasses (Fig. 5). Immobilization covaried with both soil moisture (positively) and soil temperature (negatively). Effects of vegetation types on immobilization were more clearly related to differences in soil moisture because type effects were erased when covariance with moisture was taken into account (Table 5). Although this correlative approach does not unequivocally identify moisture as the factor driving vegetation type differences in immobilization, it lends plausible support to the idea that microclimatic factors may differentially affect microbial N supply and uptake. Several other studies have shown a tendency for gross immobilization to be more sensitive to microclimate than gross production (Binkley et al. 1994, Henriksen and Breland 1999, Stottlemyer and Toczylowski 1999), although the mechanisms behind this sensitivity have not been elucidated. Increased soil moisture can directly reduce limitations to microbial metabolism and growth (Paul and Clark 1996), enhance microbe motility and access to substrates (Hamdi 1971), and increase substrate diffusion (Davidson et al. 1990). Any one or a combination of these could explain the results that we observed.

Differences in soil moisture and temperature between the grass removal treatment and the vegetation types with grasses are probably a combination of physical and biological effects of changes in species composition and vegetation structure. In the unburned forest, grasses have filled spaces between shrubs and formed a more homogenous understory canopy that reduces radiation penetration to the soil surface (Hughes et al. 1991, D’Antonio et al. 1998). Also, differences among vegetation types in native tree and shrub biomass could lead to increased soil moisture in vegetation types with grasses. Higher standing biomass of native trees and shrubs in the Woodland than in the Woodland + Grass (D’Antonio et al. 1998, Mack et al. 2001) may draw down soil water. In the Grassland, low evapotranspiration in the absence of trees and shrubs could explain higher soil moisture. Further study is needed to differentiate among these mechanisms.

Control of N mineralization by SOM composition was strongest in our system during the wet season, when microclimate was ameliorating to microbial activity. This finding is similar to that of many studies of mesic ecosystems (e.g., Wedin and Tilman 1990, Van Vuuren et al. 1992, Reich et al. 1997), semiarid ecosystems (e.g., Burke 1989), and dry ecosystems (e.g., Gallardo and Schlesinger 1995). Rates of net and gross transformations that we observed in the unburned woodland vegetation types during this season (Figs. 3–5) are similar in range to those seen in nearby mesic montane _Metrosideros_ forests (Vitousek and Walker 1989, Riley and Vitousek 1995).

During the wet season, gross NH$_4^+$ production appeared to be driving differences in net rates of N mineralization among vegetation types. Production was significantly higher in the Grassland than in the other two vegetation types. Moreover, a smaller proportion of production was consumed in this site (Fig. 5), indicating that N was available in excess of microbial demand. This indication is supported by the low pool size of mineralizable C in Grassland (Table 3), which suggests that C was relatively less available to decomposers in this site. This is probably related to lower annual rates of litter production and the higher lignin:N ratio of fresh litter in this site relative to the unburned woodland sites (Mack et al. 2001).

**Restoration of native ecosystem processes**

Understanding the relative importance of mechanisms through which plant community composition affects an ecosystem process can have important implications for the restoration of ecosystems impacted by biological invasions (Mack et al. 2001) or other anthropogenic alterations of plant composition (Keddy 1999, Chapin et al. 2000). In the case of soil N transformations, understanding the relative importance of species effects on microclimate vs. SOM quality may provide a useful dichotomy for assessing restoration and management tactics. Microclimate effects are related to the presence of the species: eradication will remove its effect on N transformations. By contrast, effects on SOM quality leave a legacy that may control N transformations and affect plant processes even after eradication.
Although our removal treatment in this study does not unequivocally recreate pre-invasion soil N dynamics, it is our best approximation in the absence of uninvaded woodland. The results from this treatment suggest that the most profound effect of exotic grasses on soil N transformations in the unburned woodland, the shift in timing of the net mineralization peak, could be mitigated by grass removal. Indeed, even 30 yr after invasion, grass C made up a relatively small percentage of the total soil organic carbon pool in the unburned woodland, and was lost from the labile pool in the two years following the removal treatment (Table 1).

In stark contrast to the unburned woodland, it seems unlikely that removal of grasses from the Grasland would mitigate their effects on N cycling, because the legacy of their effects on SOM would probably persist even if removal were feasible. The Resource Management division of HVNP has experimentally introduced several native tree and shrub species to burned areas in the seasonal submontane woodlands of HVNP (R. H. Loh, personal communication). Based on the results of our study, we conclude that reintroduction of native woody species should have several important effects on soil N dynamics. First, increased inputs of native litter to SOM should increase available C, which may decrease rates of net mineralization by increasing microbial uptake. Second, increased native woody plant biomass should result in greater synchrony between plant uptake and net mineralization, thus reducing the potential for N loss from the system. Finally, evidence from other woodland ecosystems suggests that increased woody biomass will reduce grass fuel and decrease fire spread (Doren and Whiteaker 1990, Mack and D’Antonio 1998), and slow the grass/fire cycle (D’Antonio and Vitousek 1992). In HVNP, fire return time is higher in areas that have burned once than in unburned areas (D’Antonio et al. 2000), due to changes in fuel loading and the microclimate factors that drive fire spread (Freifelder et al. 1998). We conclude that the aggressive reintroduction of native woody species into burned submontane woodland is likely to provide the most effective mitigation of the effects of exotic grasses on soil N dynamics.

ACKNOWLEDGMENTS

We thank F. S. Chapin, III, Sharon Hall, Elizabeth Holland, Pamela Matson, Edward Schuur, Wayne Sousa, and an anonymous reviewer for comments that greatly improved earlier drafts. We gratefully acknowledge the support of Peter Vitousek, David Foote, Charles Stone, and Tim Timson for providing lab facilities, site access, and logistical support. Field assistance was provided by Ruth Ley, Karen Haubensak, and Edward Schuur. Xinghua Zeng and Paul Brooks provided invaluable lab assistance. Financial support was provided by a NASA Global Change Graduate Fellowship, an NSF Dissertation Improvement Grant, and a University of California Vice Chancellor’s Research Grant to M. C. Mack, and by NSF BSR 9119618 to C. M. D’Antonio.

LITERATURE CITED


