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Grass invasion causes rapid increases in ecosystem carbon and nitrogen storage in a semiarid shrubland

ELIZABETH M. WOLKOVICH*†, DAVID A. LIPSON‡, ROSS A. VIRGINIA*, KATHRYN L. COTTINGHAM† and DOUGLAS T. BOLGER*

*Environmental Studies Program, Dartmouth College, 6182 Steele Hall, Hanover, NH 03755, USA, †Department of Biological Sciences, Dartmouth College, 6044 Gilman, Hanover, NH 03755, USA, ‡Department of Biology, San Diego State University, San Diego, CA 92182-4614, USA

Abstract

Accurately predicting terrestrial carbon (C) and nitrogen (N) storage requires understanding how plant invasions alter cycling and storage. A common, highly successful type of plant invasion occurs when the invasive species is of a distinctly different functional type than the native dominant plant, such as shrub encroachment throughout the western United States and annual grass invasions in Mediterranean shrublands, as studied here. Such invasions can dramatically transform landscapes and have large potential to alter C and N cycling by influencing storage in multiple pools. We used a manipulation of non-native annual grass litter within a shrub-dominated habitat in southern California (coastal sage scrub, CSS) to study how grass invasion alters ecosystem C and N storage. We added, removed, or left unchanged grass litter in areas of high and low invasion, then followed soil and vegetation changes. Grass litter greatly increased C and N storage in soil, aboveground native and non-native biomass. Aboveground litter storage increased due to the greater inputs and slower decomposition of grass litter relative to shrub litter; shading by grass litter further reduced decomposition of both non-native and native litter, which may be due to reduced photodegradation. Soil C and N pools in areas of high litter increased ~20% relative to low litter areas in the two years following manipulation and were generally sinks for C and N, while areas with low litter were sources. We synthesize our results into a C cycle of invaded and uninvaded areas of CSS and link changes in storage to increases in the soil fungi : bacteria ratio, increased plant inputs, and decreased litter loss. Overall, we show that grasses, especially through their litter, control important abiotic and biotic mechanisms governing C and N storage, with widespread implications for C sequestration and N storage in semiarid systems undergoing grass or shrub invasions.

Keywords: carbon cycle, coastal sage scrub, functional type, fungi : bacteria ratio, litter decomposition, Mediterranean, photodegradation, plant traits, shrub habitat

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Introduction

One of the largest sources of uncertainty in models of the global carbon (C) cycle is the role of biological feedbacks in C production and storage (Pan *et al*., 1998; Friedlingstein *et al*., 2006). Successful prediction of ecosystem C, as well as nitrogen (N), flux and storage in response to climate change requires mechanistic knowledge of how changing plant communities will influence elemental cycling and storage (Schimel, 1995; Hungate *et al*., 1996). Transformations of plant communities due to climate change have already been observed (Walther *et al*., 2002; Field *et al*., 2007), and will undoubtedly continue (Parry *et al*., 2007). In particular, climate change's synergistic effects of altered temperature and precipitation combined with increased disturbance – such as increased fire frequency or N deposition – should favor invasive plants (Walther *et al*., 2002; Field *et al*., 2007).

Plant invasions can influence biogeochemical cycling through several major pathways. Plants control above

Correspondence: Elizabeth M. Wolkovich, tel. +1 603 646 1687, fax +1 603 646 1682, e-mail: e.wolkovich@dartmouth.edu

and belowground storage in living plant materials (net primary production – NPP) (Jackson *et al*., 1996, 2002) and in dead plant matter (litter). Litter decomposition is itself strongly influenced by plant traits such as lignin and N content (Cornwell *et al*., 2008), and also by decomposer communities in certain systems (Wall *et al*., 2008). Plants also play a large role in determining soil decomposer communities (Bardgett *et al*., 1999; Wardle, 2002), which may influence storage through such properties as fungi : bacteria ratio (Kandeler *et al*., 2008).

While invasions are often associated with increased NPP and larger soil C and N pools (Liao *et al*., 2008), for some of the most large-scale invasions – shrubs into grasslands and grasses into shrublands – results are equivocal (Jackson *et al*., 2002; Bradley *et al*., 2006; Knapp *et al*., 2008). This is problematic since grasslands and savannas are the main habitat types predicted to expand in the United States due to climate change (Pan *et al*., 1998) and are prone to large-scale invasions: examples include non-native annual grasses into Mediterranean-climate shrublands globally (D'Antonio, 1993; Kalin Arroyo *et al*., 2000; Zaady *et al*., 2003), shrub encroachment in arid grasslands of the US Southwest (Schlesinger *et al*., 1990; Knapp *et al*., 2008), and the spread of *Bromus tectorum* (cheatgrass) throughout Great Basin shrublands (Chambers *et al*., 2007).

Annual grass invasions into shrublands, or the reverse, represent expansions of a functional type uncommon in the preinvasion system and are important to models of the global C cycle (Throop & Archer, 2008). We define functional type as plant species that share groupings of traits producing similarities in ecology, both in plant species' regenerative and established phases (Grime, 2001): as such, different plant functional types should dramatically alter ecosystem C and N storage by influencing soil, living plant, and litter pools. In particular, production of differing quantities and qualities of above and belowground biomass (Jackson *et al*., 2002), with associated changes in litter (Cornwell *et al*., 2008), may alter C and N turnover and storage in living plant biomass, litter and soil. While shrubs provide year-round storage in wood and their high %N litter produces 'islands' of enriched soils (Reynolds *et al*., 1999; Briggs *et al*., 2005), annual grasses produce abundant widespread litter that decomposes slowly (Cornwell *et al*., 2008), possibly allowing significant storage in litter. Additionally, grasses and shrubs may alter microclimate (Henry *et al*., 2008), which can in turn alter plant germination, growth (Boeken & Orenstein, 2001; Amatangelo *et al*., 2008), and litter decomposition (Austin & Vivanco, 2006). Finally, plant and litter quality may alter soil storage by producing soil microbial communities of differing efficiencies (Wardle *et al*., 2004). Careful consideration of multiple soil and vegetation pools and how plant functional types may control them should provide improved understanding of the impacts of grass or shrub invasions on ecosystem C and N storage.

Southern California represents an area where climate change, increased N deposition and altered fire regimes may interact with invasion by an uncommon plant functional type to impact ecosystem C and N storage. Coastal sage scrub (CSS), the dominant habitat type of California's coastal areas (Mooney, 1977), is undergoing a large-scale invasion by non-native annual European grasses. These grasses have been present in the semiarid system since at least the late 1700s, according to data obtained from mission bricks, but have increased dramatically in recent decades (Minnich, 2008). Uninvaded CSS is generally shrub-dominated with extensive bare ground and soil crusts (DeSimone & Burk, 1992), while in many invaded areas all intershrub spaces are covered by non-native grasses and their litter. In areas with highly altered disturbance regimes, CSS can type-convert to non-native grasslands with limited shrub presence (Eliason & Allen, 1997; DeSimone & Zedler, 2001).

To understand how grass invasion may alter ecosystem C and N storage in CSS, we combined a 2-year experiment manipulating non-native grass litter with extensive measurements of multiple pools and fluxes, including NPP, litter, soil pools, soil C mineralization, soil microbial communities and litter decomposition. Working in areas of high or low grass invasion, we added, left unchanged or removed non-native litter. We found rapid responses in C and N storage to altered litter cover and synthesize these findings in a C cycle for CSS which highlights the dramatic alteration due to invasion.

Materials and methods

Study site and plant types

We conducted our study within a 50 ha area of CSS located ~20 km inland in the San Diego National Wildlife Refuge, Sweetwater Unit, San Diego County, California. CSS habitat extends along the coast from northern California to Baja California, and inland >100 km at elevations <500 m, with chaparral often adjoining it at higher elevations (Mooney, 1977). It is characterized by a number of soft-leaved, drought-deciduous and evergreen sub-shrubs, and in southern California is generally dominated by *Artemisia californica* (drought-deciduous) and *Eriogonum fasciculatum* (mainly evergreen) (Schroenherr, 1992). Vegetation at the site is 56% shrub, with the remaining area covered by a mosaic of bare ground, cryptogamic crusts and mostly non-

Table 1 Native shrubs in CSS have differing plant traits relating to litter compared to the invading annual grasses

	Shrub (*Artemisia californica*)	Annual non-native grasses
Form of annual litter	Very finely divided leaves*	Stems and associated leaves
% of NPP that becomes litter	76.1†	100
%C	49.71 ± 0.69	45.01 ± 0.83
%Lignin	9.98 ± 0.46	1.39 ± 0.36
%N	1.98 ± 0.07	1.04 ± 0.08
C : N	25.60 ± 0.190	45.55 ± 2.29
Lignin : N	5.14 ± 0.46	1.32 ± 0.36

Plant tissue chemistry values are given as means ± SE and are from foliage of *A. californica* and the entire aboveground portion of grasses (*Bromus madritensis* ssp. *rubens* and *Brachypodium distachyon*). For C and N, $n = 13$ for shrubs and $n = 9$ for grasses; for lignin, $n = 3$ for shrubs and $n = 5$ for grasses.
*Schroenherr (1992).
†Gray & Schlesinger (1981).

native grasses and herbs (Morrison & Bolger, 2002) that extend to under many shrub canopies. Soils at the study site are classified as part of the friant series: shallow, well-drained, loamy, mixed, superactive, thermic Lithic Haploxerolls (66% sand, 20% silt and 14% clay) (United States Department of Agriculture: Natural Resource Conservation Service, 2008). Percent slope ranges from 3 to 40 (E. Wolkovich, unpublished data).

Differing functional types of the native shrubs and non-native grasses result in a number of different properties between the native and non-native species. We focused our study on *A. californica*, which is type-specific for southern CSS (Schroenherr, 1992). Both *A. californica* and common non-native annual grasses of CSS (predominantly *Brachypodium*, *Bromus*, and *Avena* species) have relatively shallow rooting systems (Gray & Schlesinger, 1981; Corbin *et al*., 2007), but differ in a number of their litter characteristics such as leaf size and the %NPP that becomes litter each year (Table 1).

Litter manipulation

We conducted a fully crossed, two-factor litter manipulation at the end of the growing seasons in 2005 and 2006 and followed effects through 2007. We established 56, 3 m × 3 m manipulation plots (separated by 10–50 m) around typically sized (1 m^3) *A. californica* shrubs in areas either already highly invaded by grass (>40% non-native grass cover, assessed visually) or not (<5%; factor 1, *invasion level*: premanipulation high or low invasion). We then added, removed or left unchanged grass litter (factor 2, *treatment*: addition, removal, or control) in a fully crossed design, with each of the six treatment levels (*invasion level* × *treatment*) replicated eight times. For additions we added litter to two times the natural abundance of non-native litter produced that year (see Supporting information, Table S1); for removals we cut, then removed, all non-native grass litter to within 2 cm of the ground level. We additionally had one removal-control treatment to test for artifacts of manipulation: in eight high invasion plots we removed all grass litter then immediately returned it to the plot. Abundance of non-native grasses varied considerably across the study area, such that plots in the premanipulation high- vs. low-invasion treatments were well interspersed, and not clustered into discrete areas. All plots were on east- or west-facing slopes and were of similar distance from roads (~ 0.5 km).

To measure non-native grass litter (~ ANPP) in naturally invaded areas for 2 years (2005–2006) and allow estimation of litter removed or added per plot for the experiment, we estimated total litter in 9 m^2 high invasion areas in 2005 and 2006. Working outside of experimental plots, we measured how many 0.025 m^3 units of litter were in a 9 m^2 area, then collected and sorted three units of the litter (total of 0.075 m^3) in the lab to species, dried (60 °C for 72 h), and weighed.

Soil sampling

We sampled soil to assess soil organic C (SOC), labile C, total N (TN), and the microbial community (i.e. fungi : bacteria ratios). We took three cylindrical soil cores per plot (3 cm wide, 10 cm deep, one 1–5 cm from inside the dripline of shrub, and two 5–20 cm outside the shrub dripline, we removed the litter layer – generally herbaceous as *A. californica* shrubs do not usually develop a litter layer – before taking cores) in midgrowing season both before and after the manipulation (29 April 2005 and 13 March 2007). We placed samples in a cooler in the field and kept them at 3 °C until analysis. Samples were sieved through 2 mm mesh to remove roots and rocks; fine roots were removed with forceps. We measured SOC and TN on dried, ground samples by elemental analyzer (Carlo Erba NA 1500 series 2, CE Elantech Inc., Lakewood, NJ) and standard protocols (Sollins *et al*., 1999). In 2007, we determined the labile C pool and C mineralization rates through lab incubations: respiration of ~6.5 g of soil at 15% gravimetric water content (GWC) was measured every 3–5 days for 60 days (Robertson *et al*., 1999), using 1 mL gas samples measured on an infrared gas analyzer (CI-301, CID Inc., Vancouver, WA, USA). We then calculated the size of the labile C pool (C_l), decay rate constant of the labile

pool (k_l), and mineralization rate of the recalcitrant pool (k_r) for each soil sample using a two-pool model (McLauchlan & Hobbie, 2004). We focus on k_l and C_l, which can be robustly predicted from short-term C mineralization studies (McLauchlan & Hobbie, 2004). In 2007, thoroughly mixed (not sieved) subsamples of soil from 24 plots (including five treatments, all but low invasion × removal, 3–5 replicates per treatment) were shipped immediately upon leaving the field to Soil Food Web Inc. (Corvallis, OR, USA) for total fungi (TF) and total bacteria (TB) biomass ($\mu g\, g^{-1}$ soil) analysis by microscopy and biovolume methods (Ingham *et al*., 1986a, b, 1991).

Litter decomposition

We measured non-native and native litter decomposition with a 23-month litterbag study. Fiberglass litterbags (0.5 mm mesh, 10 cm by 10 cm) were filled with 10 g of litter of either the native shrub *A. californica* or a mix of non-native grasses (45% *Brachypodium distachyon*, 20% *Avena barbata*, 15% *Vulpia myuros*, 10% *Bromus madritensis* ssp. *rubens*, 10% *Bromus hordeaceus*; amounts based on natural abundance at the study site in 2005). We placed all litterbags on the soil surface ~10 cm outside of the dripline of the focal shrub following the manipulation in 2005 and retrieved them after 6, 12, and 23 months. After retrieval, we dried litter at 60 °C for 72 h, then weighed to determine dry mass remaining. We determined %C and %N on a ground subsample (Carlo Erba NA 1500 series 2 elemental analyzer) (Sollins *et al*., 1999). We estimated the overall litter decay rate (k) with a simple first order equation and calculated litter N as %N × litter mass remaining.

Tissue chemistry

In May 2004, we clipped aboveground *A. californica* foliage and two common non-native grasses (*Brachypodium distachyon* and *Bromus madritensis* ssp. *rubens)* for tissue analysis of %C and %N (as described above) and for %lignin by acid detergent fiber (ADF) analysis (Custom Lab Inc., Golden City, MO, USA).

Vegetation sampling

We measured ground cover by recording cover at 18 points/plot as either grass litter or native/bare ground cover. Points were located every 0.5 m along two diagonal transects across each plot. We divided total hits by 18 to obtain percent ground cover. In addition to litter cover we assessed plant density by recording at each of the 18 points plants, by species, to intersect the point intercept line, with a maximum of three hits per 0.1 m per species. We converted the data to a plant density index out of 100 by dividing plant hits by the maximum number of hits of all species of any one plot each year.

Aboveground native shrub biomass was estimated by clipping a small section (~5%) of the shrub, then visually estimating the number of similarly sized branches present on the shrub. *A. californica* shrubs have no central stem and this method has been found to be quite robust compared with volume or other estimates (Wolkovich, in press). We separated, dried and weighed current (foliage and nonlignified stem) growth and growth from previous years' growth (mostly wood) in the lab. We converted this to mean shrub NPP per experimental ($9\, m^2$) plot using our %C of shrub foliage and known shrub cover for our study site (Morrison & Bolger, 2002).

Statistical analyses

We conducted analyses using JMP 5.0 (SAS Institute Inc., Cary, NC) and R 2.5.1, including the packages car, multcomp, and nlme (R Development Core Team, 2007). We used correlation and regression analyses to relate soil %C and %N to non-native litter cover; this exploited the nearly continuous variation in non-native litter cover that arose from our manipulations, premanipulation variability, and interannual variability (low litter production in 2005 due to previous dry years vs. high litter years in 2006 and 2007 following high rainfall of 2005 season). We used two-way ANOVA to analyze the labile C pool (C_l, $\log_{10}$-transformed) and its decay rate (k_l) as a function of invasion level, treatment, and their interaction (our experimental design). We used mixed-effects general linear models (GLM) to analyze our litter data with plot as a random effect (nested within invasion level and treatment). To analyze litter decomposition rates (k, $\log_{10}-$transformed) and litter loss in the first 6 months we included in the GLM our two-way experimental design and litter type (native vs. nonnative). We further examined significant treatment effects in these models with Dunnett's post-doc test, which provides comparison with controls. Because litter N appeared to decay linearly, we analyzed it with a model that included our experimental design, litter type (native vs. nonnative) and 'months in field' (0, 6, 12, 23), including all possible interactions. We analyzed only a subset of our treatments for TF : TB and thus we used a one-way ANOVA combined with *a priori* linear contrasts designed for the experiment plus a contrast of high vs. low invasion. *A priori* contrasts were: high invasion × control vs. high invasion × removal (contrast 1) and low invasion × addition vs. low invasion × control (contrast 2). We demonstrated that removal effects were due to litter removal and not

artifacts of the manipulation by one-way ANOVA or GLMs (see Table S2).

Summarizing effect of invasion on the C cycle

We compared overall changes in the C cycle due to grass invasion in terms of C pools ($g\,m^{-2}$) and fluxes ($g\,m^{-2}\,yr^{-1}$) using data from high and low invasion control plots. Data originally expressed g^{-1} soil were converted using a bulk density of $1.46\,g\,cm^{-3}$ for low invasion and $1.24\,g\,cm^{-3}$ for high invasion areas (D. Lipson, unpublished data) and a 10 cm sampling depth, which includes the A soil horizon and part of B (United States Department of Agriculture: Natural Resource Conservation Service, 2008).

We estimated mean NPP ($g\,C\,m^{-2}$) from our aboveground measurements of native *A. californica* NPP and non-native annual grass NPP (averaged across 2005 and 2006). We estimated root production in non-native annuals assuming root : shoot ratios of 0.95 (based on studies of *B. madritensis* ssp. *rubens* and *Brachypodium retusum*) (Johnson & Lincoln, 1991; Caturla *et al*., 2000). Standing belowground biomass (live plus dead) of annuals was calculated by dividing root NPP by published turnover rates for grasslands (0.52 year, Gill & Jackson, 2000). For native shrubs we used a root : shoot ratio of 1.2 (Jackson *et al*., 1996) to calculate standing root biomass, and then determined root NPP for shrubs by multiplying the calculated standing root biomass by turnover rates (Gill & Jackson, 2000).

These mean NPP values, combined with known differences in %NPP that becomes litter (Table 1) were used to calculate litterfall. We predicted standing aboveground litter pools in the uninvaded and invaded systems at steady state by dividing litterfall rates by the observed litter decomposition rate constants. We calculated litter breakdown by photo- and biological degradation by multiplying the steady-state litter pool by the decomposition rates obtained during the 6 months summer-fall (dry) or the 6 months winter–spring (wet) periods of our litterbag study, respectively.

Total measured SOC minus labile C gave the size of the recalcitrant C pool. The rate of C leaching value (measurement of water-extractable C from litter) from the litter to the soil component was estimated by multiplying the litterfall rates by the percent water-soluble C of the two litter types (16% for grasses, 32% for native shrubs; D. Lipson, unpublished data).

To estimate annual soil respiration by various pools, we combined our C mineralization data with field respiration measurements and a model of annual respiration developed for Diegan CSS. We combined respiration data taken from experimental plots in 2007 with observed effects of soil moisture, vegetation cover (bare ground and soil crust, under shrub canopy, under non-native grass cover), and temperature on soil respiration (Lipson *et al*., unpublished data). This model is based upon extensive multiyear measures and analysis of CSS soil respiration. During the growing season, the model simulates respiration from soil moisture using separate regressions for the varying vegetation covers. During the early winter, the model is based on soil temperature. It is thus sensitive to both moisture (15% decrease in precipitation leads to 9–11% declines in annual respiration) and temperature (increase of 3 °C in winter temperature leads to 18–20% increases) (Lipson *et al*., unpublished data). Daily temperature and soil water estimates were simulated from field observations (Wolkovich *et al*., unpublished data). The proportional cover of each vegetation type was estimated from our vegetation data from control plots as 42 : 50 : 8 vs. 29 : 2 : 69 for shrubs : grasses : ground in invaded vs. noninvaded plots. We estimated contributions to soil respiration from the turnover of the labile and recalcitrant C pools by scaling from the laboratory conditions under which the rates were observed (15% GWC, 22 °C) using the same soil respiration model. Root respiration was assumed to be the difference between total soil respiration and microbial respiration of the labile and recalcitrant C pools. Litter respiration was estimated from our litterbag study as biological degradation rate minus leaching.

Finally, we estimated gross primary productivity (GPP) by adding above and belowground NPP, with root respiration (Chapin *et al*., 2002). This estimate does not include root exudation, stem respiration, or erosional C losses.

We estimated standard errors for calculated quantities by propagating errors from the individual components according to standard rules (Taylor, 1997). When standard errors were not available from the literature (Jackson *et al*., 1996; Gill & Jackson, 2000), the reported values were applied as constants. In one case (Gray & Schlesinger, 1981), errors for NPP and litterfall were not provided in the original publication but were calculated from information therein (relative errors were 13% and 5%, respectively).

Results

Soil

Soil C (SOC) and N (TN) were positively related to non-native grass litter both before (2005: Fig. 1, see legend for correlation statistics) and after the manipulation (2007 relationship of litter cover and soil C: $r = 0.38$, $t_{49} = 2.13$, $P = 0.04$, litter cover and N: $r = 0.24$, $t_{49} = 1.73$, $P = 0.09$). Moreover, soil C and N changed

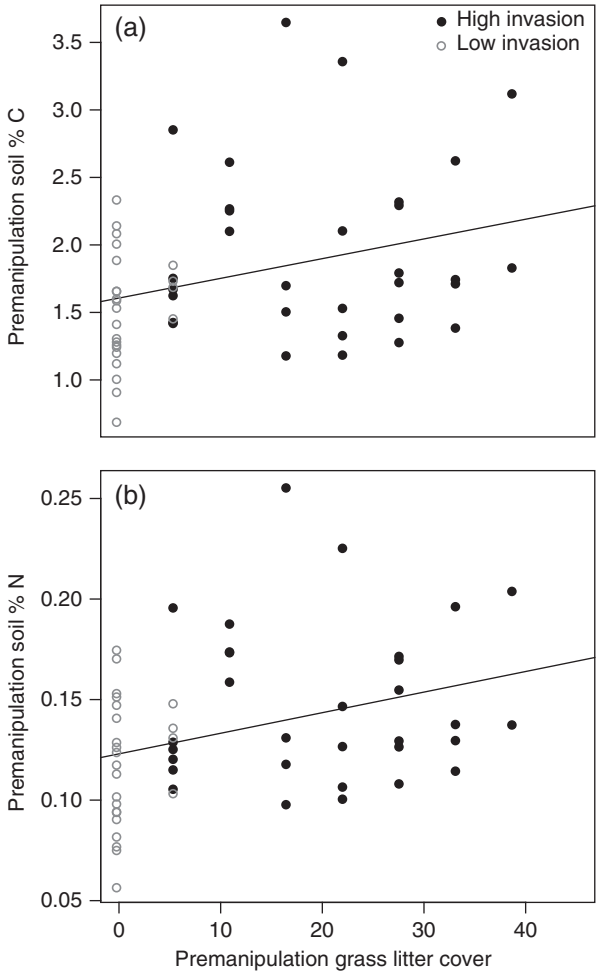

Fig. 1 Before the litter manipulation (2005), soil % C (a, $r = 0.32$, $t_{53} = 2.40$, $P = 0.02$) and soil % N (b, $r = 0.34$, $t_{53} = 2.60$, $P = 0.01$) were positively correlated with non-native grass litter cover. Black solid symbols represent high invasion areas, while gray open symbols represents low invasion. Soil C : N ratio was consistently 13.1 ± 0.1 (mean ± SE) across plots.

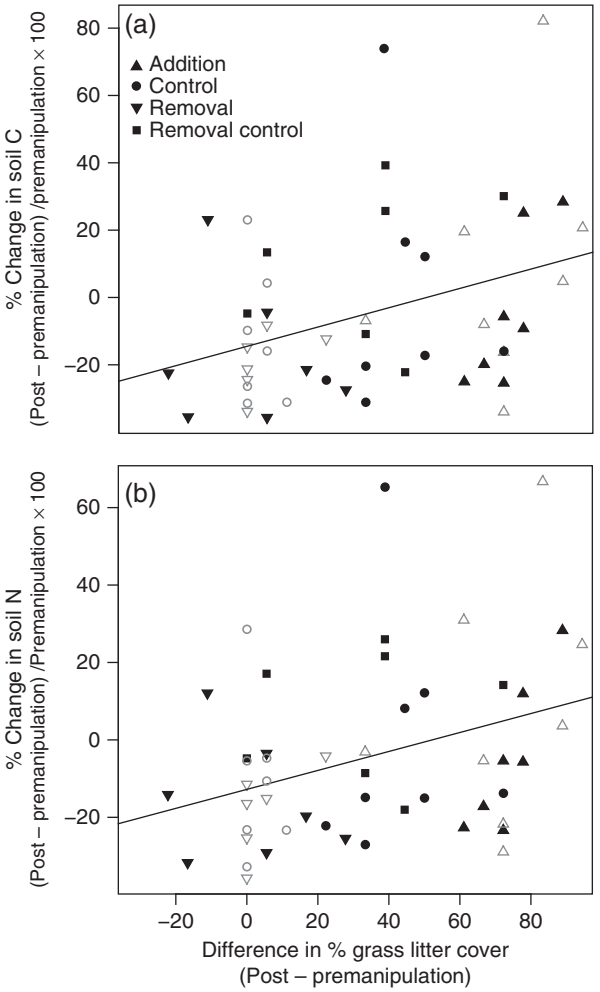

Fig. 2 The % change in soil C (a) and N (b) from 2005 to 2007 was a linear function of change in non-native litter due to the manipulation. Black solid symbols represent high invasion areas, while gray open symbols represent low invasion; symbol shapes represent litter treatments. % Change soil C = 0.28 litter cover −14.20, $t_{48} = 2.54$, $P = 0.01$; for N: % change soil N = 0.24 litter cover −12.54, $t_{48} = 2.49$, $P = 0.02$, $R^2 = 0.12$ for both. These results did not depend on the two most extreme (>60%) increases (analyses without those points: % change soil C = 0.20 litter cover −14.66, $t_{46} = 2.24$, $P = 0.03$, $R^2 = 0.10$; for N: % change soil $N = 0.17$ litter cover −13.00, $t_{46} = 2.18$, $P = 0.03$, $R^2 = 0.09$). Slopes for C and N were not different (comparison of slopes test: $t_{96} = 0.28$, $P = 0.78$) and the soil C : N ratio was consistently 13.0 ± 0.1 (mean ± SE).

in response to litter during our manipulation period, with many plots increasing or decreasing by 20% (Fig. 2).

High invasion areas had 45% greater mean labile C pools than low invasion areas (Fig. 3a). Additions caused increases in labile C, while removals did not cause decreases (Fig. 3a). In contrast, decay rate constants for the labile pool (k_l) decreased with litter addition in both low and high Invasion areas (Fig. 3b). However, decay rates were unchanged by litter removal (Fig. 3b).

Microbial community biomass became more fungal-dominated with increased litter (Fig. 3c). TF : TB biomass was greater in high invasion areas (contrast of high invasion treatment vs. low invasion treatments: $F_{1,16} = 8.4$, $P = 0.01$). Additions tended to have higher TF : TB than controls (Fig. 3c), although these differences were not statistically significant (contrast 2: $F_{1,16} = 2.06$, $P = 0.17$). Removals from high invasion areas were not significantly different from controls in high invasion areas (Fig. 3c, contrast 1: $F_{1,16} = 0.27$, $P = 0.61$). Changes in the microbial community composition were not associated with changes in size of the total microbial pool (one-way ANOVA of mass of

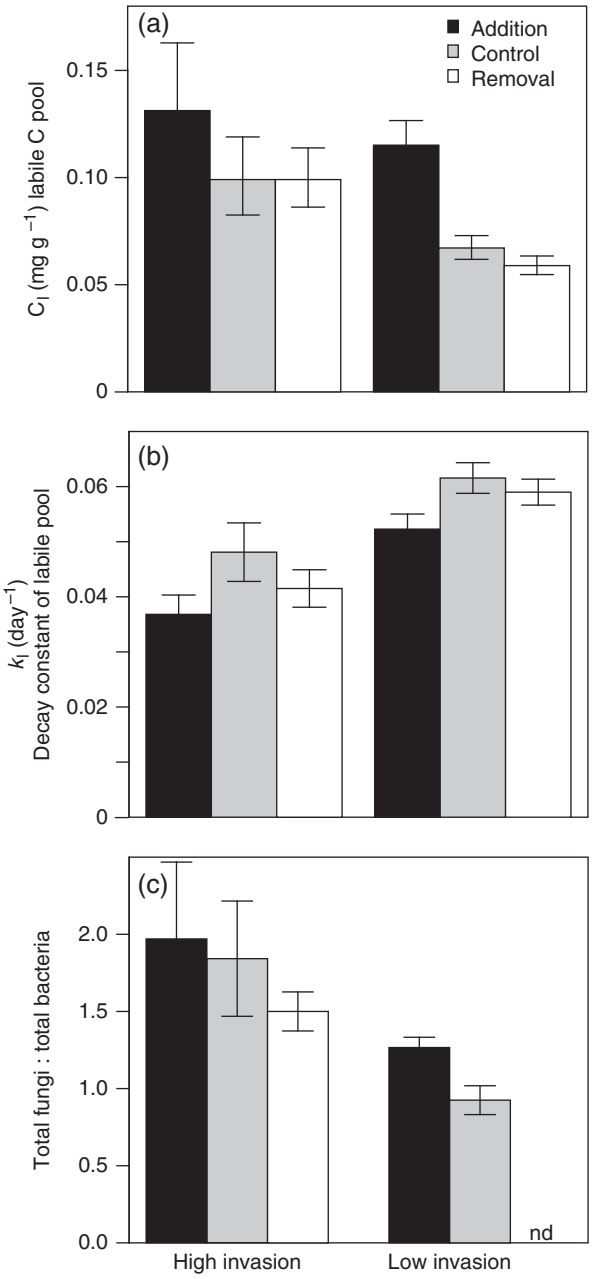

Fig. 3 Two years after the manipulation, the labile C pool (a), its decay rate (b), and the total fungi : total bacteria ratio (c) all varied due to litter manipulation. The labile C pool ($F_{2,36} = 6.54$, $P < 0.001$) and labile C decay rate ($F_{2,36} = 4.67$, $P = 0.02$) varied by litter treatment. Labile C pools increased with litter addition (a, Dunnett's test: control vs. addition, $P = 0.01$, control vs. removal, $P = 0.84$) and were greater in premanipulation high invasion areas ($F_{1,36} = 8.35$, $P < 0.01$), while decay constants for the labile C pool decreased with litter addition (b, Dunnett's test: control vs. addition, $P = 0.007$, control vs. removal, $P = 0.29$) and were lower in high invasion areas ($F_{1,36} = 24.42$, $P < 0.0001$). There was no interaction between invasion level and treatment ($P > 0.5$). Grass invasion caused a shift in the microbial community, becoming more fungal-dominated (c), see text for statistics. 'nd', indicates that we did not collect data for that treatment. Scale bars are means ± 1 SE.

Table 2 General linear model explaining total (23 months) litter decomposition (k) and mass loss after only 6 months due to our experiment (invasion level and treatment, abbreviated here from addition, removal or control) and litter type. Native litter was Artemisia californica leaves, while non-native litter was a mix of non-native annual grasses. See also Fig. 4

	Litter k			Mass loss after 6 months	
GLM:	*df*	*F*	*P*	*F*	*P*
Invasion level (high/low)	1	0.06	0.80	9.11	0.004
Treatment (add/con/rem)	2	5.60	<0.005	11.52	<0.0001
Litter type (native/non-native)	1	199.00	<0.0001	101.37	<0.0001
Invasion level × treatment	2	3.21	0.05	1.20	0.31
Invasion level × litter type	1	0.14	0.71	3.08	0.09
Treatment × litter type	2	0.63	0.54	2.50	0.10
Invasion level × treatment × litter type	2	1.22	0.31	1.68	0.20
Error	39				

fungi + bacteria: $F_{4,4} = 1.12$, $P = 0.38$; all contrasts non-significant at $\alpha = 0.05$).

Non-native and native production

Non-native litter produced annually in unmanipulated areas of high invasion was 271 ± 16 (mean ± SE) g m^{-2} in the first year of the manipulation (2005), an above-average rainfall year (521 mm, 190% of the 50-year mean). In 2006, a slightly below-average rainfall year (74% of the mean), litter was 68 ± 5 g m^{-2}. Because the litter manipulation was based on litter produced each year, in 2005 the manipulation was approximately 4 × larger than in 2006 (Table S1).

Annual production by the native shrub responded strongly to litter treatments ($F_{2,41} = 7.81$, $P = 0.001$) with shrubs in addition plots producing nearly 3 × as much new growth as those in removal plots (190 ± 22 vs. 65 ± 13 g). Production was also greater in high invasion areas ($F_{1,41} = 4.61$, $P = 0.04$), and was positively related to previous (woody) growth ($F_{1,38} = 53.48$, $P < 0.0001$). Previous woody growth itself varied by invasion level ($F_{1,52} = 22.50$, $P < 0.0001$) with 370 ± 23 g shrub^{-1} in high invasion vs. 189 ± 14 g shrub^{-1} in low invasion areas.

Litter quality and decomposition

Litter quality differed with metric. Grass litter had lower lignin : N ratios than the native shrub ($F_{1,6} = 170.0$, $P < 0.0001$, Table 2). However, grasses had higher C : N than the native shrub ($F_{1,20} = 40.4$, $P < 0.0001$, Table 2).

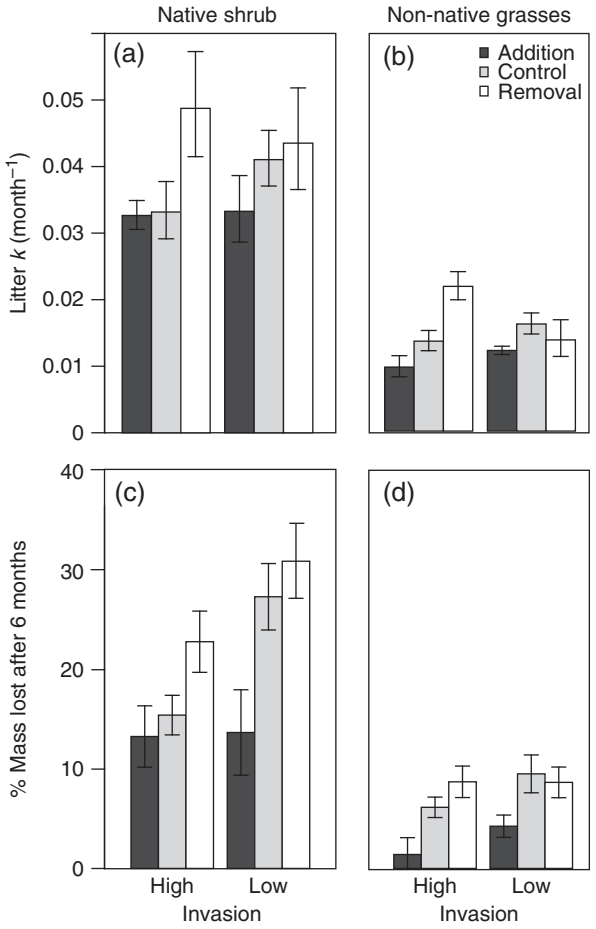

Fig. 4 Total litter decay rate (k) over 23 months (a, b) and litter decomposition within the first 6 months (c, d) varied by litter type and due to the litter manipulation. Mean litter decomposition rates were greater for native (left) litter than for non-native (right) litter (a, b). Further, litter in high invasion areas decayed more slowly than in low invasion areas and litter treatments altered decomposition, with greater decomposition in removal plots (Dunnett's test: control vs. removal, $P = 0.12$), and lower decay in addition plots (Dunnett's test: control vs. addition, $P = 0.07$). Trends were similar for mean mass lost after the first 6 months in the field (c, d), a period of little to no microbial activity (Dunnett's test: control vs. addition, $P = 0.002$, control vs. removal, $P = 0.20$). Scaled bars are means ± 1 SE. For statistics see Table 2.

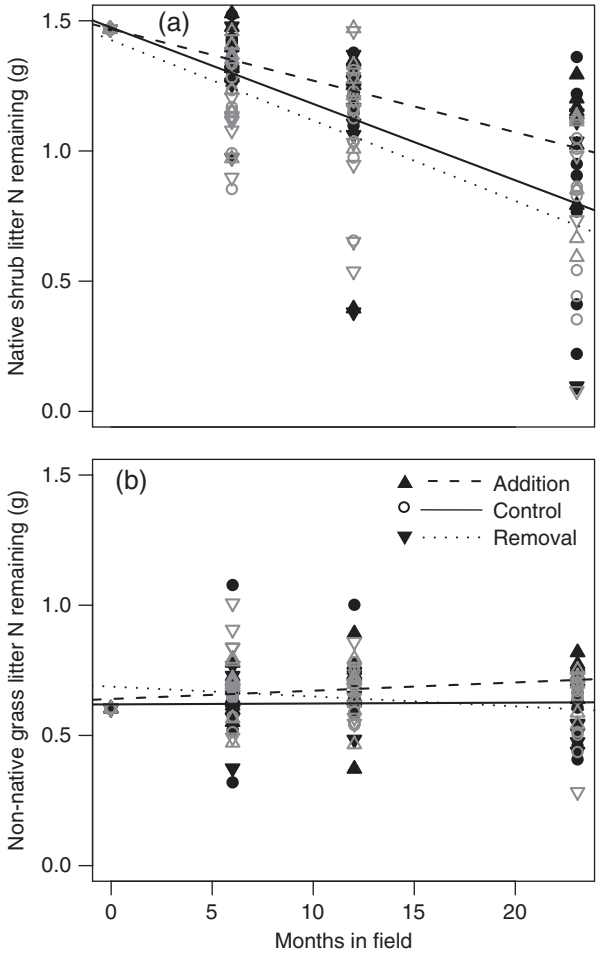

Fig. 5 Responses of native (a) and non-native (b) litter N to months in field varied due to the litter manipulation. N declined sharply in native litter over months in field (a), while N in non-native litter did not (b). Addition treatments of native litter declined less than control and removal treatments (a). Filled, black symbols represent high invasion, while open, gray symbols represent low invasion plots. For statistics see text.

Non-native litter decreased litter decomposition (Fig. 4a and b) in two ways. First, non-native grass litter decomposed more slowly than native shrub litter (Table 2: litter type, $P < 0.0001$). Second, addition of non-native litter decreased decomposition of both native and non-native litter (Table 2, Fig. 4a and b), while its removal tended to increase decomposition (Fig. 4a and b). The effect of litter manipulation was marginally dependent on invasion level (Table 2), where treatments that reinforced the premanipulation condition – additions to high invasion areas and removals from low invasion areas – showed smaller effects (Fig. 4a).

We found striking differences in litter mass lost after only 6 months (Fig. 4c and d), which encompassed the summer and fall dry season – a period of expected little or no microbial activity (D. Lipson, unpublished data). Invasion level, treatment and litter type all showed that the presence of non-native litter cover decreased litter mass lost (Fig. 4c and d, Table 2). Additions of litter decreased decomposition while removals did not alter decomposition (Fig. 4c and d).

Non-native litter influenced the loss of N from litter during the 23-month litterbag study (Fig. 5a and b). Less

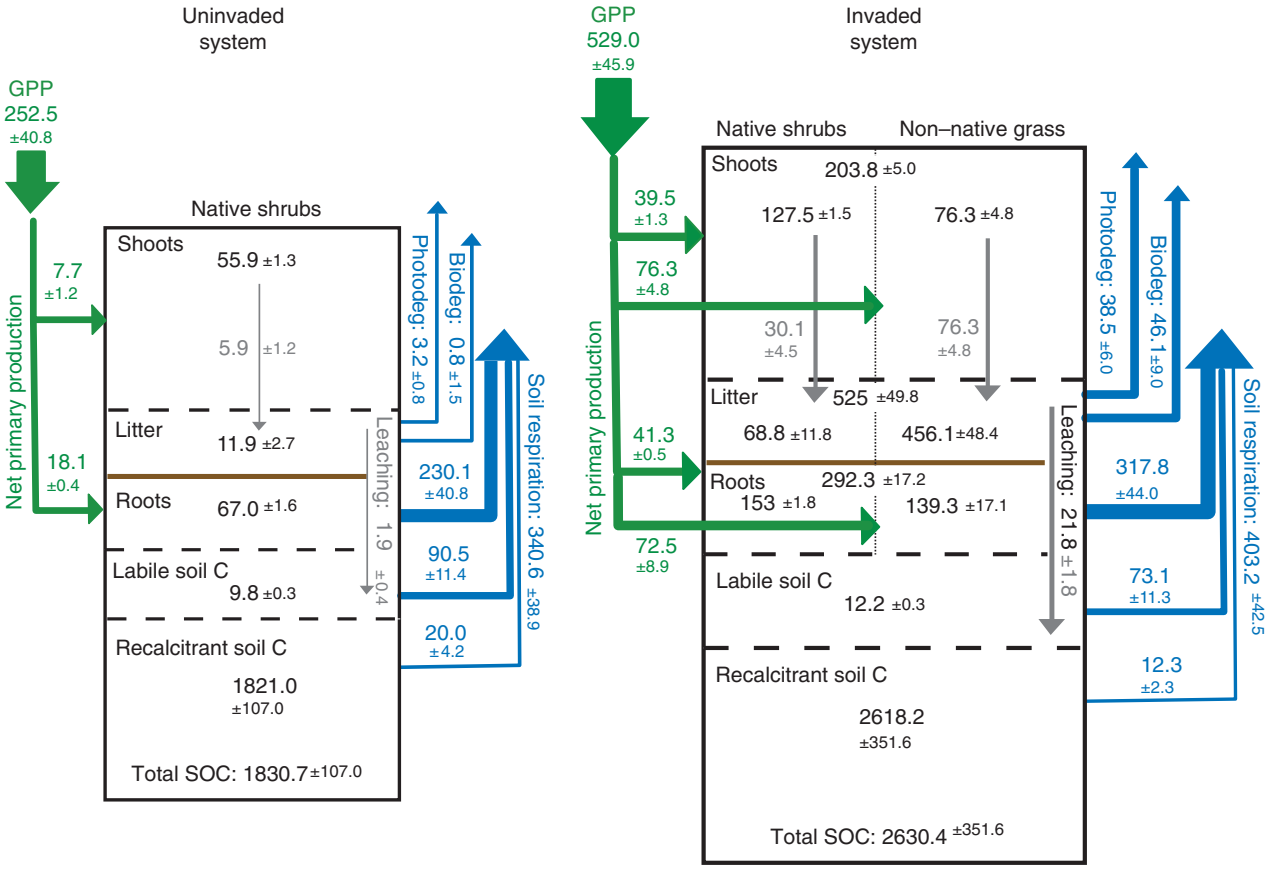

Fig. 6 The C cycle in CSS ecosystem with and without invasion by non-native grasses. Pool sizes (shown in black, ± 1 SE) are in units of g m^{-2}. Fluxes (shown with arrows in blue, green and gray; all, ± 1 SE) are in units of g m^{-2} yr^{-1}. 'Shoots' refers to aboveground live plant biomass. Values for litter represent steady-state estimates. 'Leaching' represents soluble litter C transported belowground. Losses of litter C to the atmosphere through photodegradation and biodegradation are also shown. Soil respiration is divided into fluxes from roots, and the labile and recalcitrant C pools. For detailed procedures see Materials and Methods. The thick brown line delineates above from belowground.

N was lost from non-native grass litter than native shrub litter (Fig. 5; litter type × months: $F_{1,288} = 152.49$, $P < 0.0001$; litter type: $F_{1,288} = 1096.15$, $P < 0.0001$). N loss also varied by litter treatment (treatment: $F_{2,41} = 9.24$, $P < 0.001$), with addition of non-native grass litter tending to decrease N loss in comparison with removal and control treatments (Fig. 5a and b), though the magnitude of these effects varied by litter type ($F_{2,288} = 3.36$, $P = 0.04$). The influence of litter type on N loss also depended on its interaction with invasion level ($F_{1,288} = 10.18$, $P = 0.002$) due to a minor decrease in loss in high invasion areas for native litter (Fig. 5a). All other interactions were non-significant ($P > 0.1$).

Overall changes to the C cycle

Overall, the highly invaded system was ~9× more productive (greater above and belowground NPP) and had increased ecosystem C storage in litter and soil (1.4×) compartments (Fig. 6). The increased plant biomass in the highly invaded system resulted from increases in both native shrub and especially non-native grass growth (Fig. 6). The predicted steady-state litter pool increased 44-fold and this increased litter pool provided more substrate to leach into the labile soil C pool. The modeled portion of soil respiration attributed to roots increased by ~34% with invasion, which is consistent with the expected increase in root biomass and production. In contrast, microbial respiration decreased in the invaded system, due to the decreased turnover rates of the labile and recalcitrant soil C pools. Our C cycle values were consistent with numbers from other studies of semiarid grass and shrublands (see Table S3), and uncertainties in most pool sizes and flux rates were relatively small (SE generally <15% calculated mean value).

Discussion

Grass invasion greatly increased both ecosystem C and N storage through dramatic increases in litter and soil pools (Fig. 6). Importantly, soil C and N responded to altered non-native litter within 2 years after the manipulation; this is striking because SOC pools generally turn over on decadal or longer time scales (Baisden *et al*., 2002; Taneva *et al*., 2006). Our results demonstrate that increased plant inputs and decreased losses can cause rapid changes. In particular, we link changes to increased grass litter, which led to a more fungal-dominated microbial community, decreased photodegradation, and increased NPP.

System productivity increased due to increased non-native and native plant biomass. Overall aboveground NPP increased 15-fold due to increased growth of native shrubs and especially non-native grasses (Fig. 6) in the interstitial spaces between shrubs, which accounted for $\sim$70% of this increase; in contrast, uninvaded systems were dominated by cryptogamic crusts and bare ground resulting in much lower NPP. Further, our estimates of NPP, based on standing crops near the end of the growing season, may be low, as the non-native annual grasses we studied have high seedling thinning early each growing season (Eviner & Firestone, 2007). Thinning provides highly labile substrate for active microbes and may contribute to greater SOC and N stabilization (Eviner & Firestone, 2007).

Storage in soil

Our finding that soil C and N changed equivalently (Figs 1 and 2) supports the idea that the increased NPP contributed greatly to observed soil changes, as opposed to changes by other processes such as N fixation that would influence one pool independently. In addition to increased NPP, changes in soil C and N pools are likely also due to decreased loss and a more efficient microbial community.

Shifts in the microbial community towards increased fungal domination, as we observed with invasion (Fig. 3c), can increase soil C storage (Kandeler *et al*., 2008). Fungi are generally more efficient, respiring less per unit of biomass than bacteria (Lipson *et al*., 2005; Joergensen & Wichern, 2008; Lipson *et al*., 2008) and thus are often linked to greater C storage (Kandeler *et al*., 2008; Persiani *et al*., 2008). This shift to a more efficient fungal-dominated microbial community is consistent with our findings of decreased decay rates of the labile soil C pool (Fig. 3b) and the estimated declines in microbial respiration following invasion (Fig. 6). The observed increases in soil respiration with invasion appear to be due to increased root biomass and associated root respiration rather than microbial respiration of soil C, and therefore do not offset the gross addition of plant matter to the soil C pool.

The stimulation of fungi by non-native grass litter is likely explained by the litter's relatively high C : N ratio, as high C : N organic matter and lower N availability generally favor fungi (de Vries *et al*., 2006; Hogberg *et al*., 2007; Joergensen & Wichern, 2008). Soil fungi may also benefit disproportionately from litter addition owing to their filamentous morphology, which should allow them to colonize litter while simultaneously exploiting moisture and other soil resources. The fungal response to non-native grass litter was likely due to saprotrophs, as previous work in CSS has shown that the common non-native grass *B. madritensis* harbors a higher abundance of non-mycorrhizal fungi than the dominant native shrub (*A. californica*), with no difference in arbuscular mycorrhizal spore density between the two species (Siguenza *et al*., 2006).

Our results suggest grass invasion may act to switch a system that is typically a variable C source – depending on rainfall – to a sink (Hastings *et al*., 2005). Over the two below-average rainfall years of our experiment, areas of low non-native litter lost C and N (Fig. 2), indicating that, at least in some years, CSS soils may be a C source to the atmosphere or areas lower on the landscape. In contrast, soils in areas of non-native litter cover tended to be sinks, indicating the increased litter layer in the invaded state may have protected the soil surface and slowed loss rates, contributing to C and N sequestration and our observed larger C and N pools (Renard *et al*., 1997). Reduced erosional losses may also play a role in soil C and N storage: CSS tends to form on steep slopes and, in uninvaded habitat, has an open canopy structure with extensive areas of exposed soil (Mooney, 1977; Schroenherr, 1992). In steeply sloped areas of a similar watershed, erosional losses of C represented about 3% of NPP (Smith *et al*., 2007).

According to our C cycle, the recalcitrant C pool was $\sim 800\,g\,m^{-2}$ (44% greater) in the invaded system: increased root production, reduced microbial respiration and reduced erosional C losses likely explain this increase. Increased root production would contribute $\leq 95.7\,g\,m^{-2}\,yr^{-1}$, depending on the portion of root turnover that enters the recalcitrant C pool, while reduced microbial respiration would contribute a relative gain of $25.1\,g\,m^{-2}\,yr^{-1}$, compared with the uninvaded state. Additionally, aboveground litterfall could represent a large flux into the recalcitrant soil C pool: insect activity may mix material into the soil, and otherwise labile soluble material leached from the litter could become stabilized by interactions with minerals (Torn *et al*., 1997).

The respiration flux we calculated from the labile C pool implies an equivalent source of labile C input to the soil. Leaching of soluble C from aboveground and

belowground (root) litter would contribute substantially to the labile C pool in the invaded state (30%), but less so in the uninvaded state (2%). Values of nonstructural carbohydrate in roots generally range from 5% to 10% for grasses (Dormaar & Willms, 1993; Turner *et al*., 2006), and 7.5–15% for shrubs (Cruz & Moreno, 2001; Olano *et al*., 2006; Palacio *et al*., 2007); therefore, root turnover could account for up to 3% and 18% in the uninvaded and invaded states, respectively. Root exudation is one possible flux that may be very important to C cycling but can be difficult to measure in the field (Kuzyakov & Domanski, 2000). In the uninvaded state a rate on the order of 34% of GPP (within the range of measured C exudation by plants (Heulin *et al*., 1987; Hamilton & Frank, 2001)) would be sufficient to explain the remaining flux of labile C, while the invaded state would need only 6.6%. It is also likely that some flux of labile C derives from the recalcitrant pool, through processes such as cellulose degradation.

Storage in litter

Ecosystem C and N storage also increased due to greater litter accumulation, as a result of increased NPP, a higher proportion of NPP falling as litter, and 2.7× slower decomposition of non-native vs. native litter (Figs 4 and 5). The decreased decomposition of non-native litter relative to native litter in the same litter treatments (Fig. 4) was presumably due in part to its lower quality for microbes (Cornwell *et al*., 2008). The high C : N of non-native litter compared with native litter would be expected to slow decomposition (Rousk & Baath, 2007), and this was apparently more consequential than the low lignin content of non-native grass litter. There may also have been some mechanical effects from decreased surface area : volume ratios (Maloney & Lamberti, 1995): non-native grass litter was dominated by large stems while *A. californica* litter was mainly small leaves (Table 1). Reciprocal litter transplant studies could further test if there were effects due to the microbial system being primed to decay its own litter (Rothstein *et al*., 2004; Schmidt & Lipson, 2004; Strickland *et al*., 2009).

Non-native litter cover decreased decomposition of both its own and native litter by >40% (Fig. 4). We suggest this may be predominantly due to decreased photodegradation. In the first 6 months of our litterbag study there was no rainfall, no detectable microbial activity (D. Lipson, unpublished data), and extremely low ground arthropod activity (E. Wolkovich, unpublished data); however, we measured significant mass loss of native and non-native litter in areas with low litter cover. These losses may be caused by photochemical (UV) breakdown (Austin & Vivanco, 2006), as losses from wind were limited due to our litterbag design. In plots with a thick grass litter layer, litter would have been protected from photodegradation by shading from overlying grass litter. Photodegradation is a dominant cause of litter decomposition in many semiarid systems and our initial 6 months loss rates of ~50% are similar to short-term estimates of photodegradation loss in other semiarid habitats (Austin & Vivanco, 2006; Henry *et al*., 2008). However, without direct manipulation of solar radiation we cannot be certain changes were not due to other factors such as temperature (Brandt *et al*., 2007) or soil infiltration (Throop & Archer, 2007). Additionally, our litterbag design may have prevented more photodegradation than studies using open-top litter trays (e.g. Austin & Vivanco, 2006). Still, our 6 months loss rates and differences in litter treatments suggest photodegradation may be important and warrant further study. Photodegradation results in a 'short-circuit' of the C cycle as organic matter is lost to the atmosphere (Austin & Vivanco, 2006). Therefore, non-native grass litter's role in disrupting photodegradation provides a major mechanism to prevent C and N loss from the system. Litter not photodegraded in the arid months is then available for decomposition during the growing season when it is more likely to be stabilized in the soil.

Storage in other pools

Although we measured C and N storage in soil, biomass and litter, there are several pools we did not explicitly measure. To estimate belowground root biomass, we relied upon our aboveground measurements and published ratios, some of which did not include error estimates (Jackson *et al*., 1996; Gill & Jackson, 2000). We did not completely assess belowground wood storage, but did measure aboveground standing wood biomass in shrubs, which was much greater in invaded areas. Because we found increased shrub stem growth, we expect this pool also increases with invasion. Additionally, we found that even if non-native grasses completely displaced the shrubs, this loss of all C in woody tissue would be more than compensated for by the increase in litter and soil C. Finally, we constrained our soil analyses to the top 10 cm, although deeper reservoirs of substantial C and N pools have been found in other systems (Jackson *et al*., 1996; Jobbagy & Jackson, 2000). However, soil surveys from our study area do not support the presence of deep organic matter pools (United States Department of Agriculture: Natural Resource Conservation Service, 2008).

Implications for grass ↔ shrub conversions and invasions

Our results provide experimental evidence that ecosystems converting between functional types undergo rapid

alterations of C and N storage and support findings that the mirror invasion – shrub encroachment into grasslands – decreases C storage (Jackson *et al*., 2002; Knapp *et al*., 2008). Additionally, our results suggest that similar mechanisms related to soil microclimate may cause storage changes in CSS and shrub encroachment systems (Schlesinger *et al*., 1990). In CSS, non-native grass litter increases soil moisture and decreases soil temperature (Wolkovich *et al*., unpublished data), which may account for the observed increases in soil storage; the reverse – decreased soil moisture, increased soil temperature, and associated declines in soil storage – is seen in shrub invasions (Schlesinger *et al*., 1990).

However, our results contrast with findings of decreased C and N storage in cheatgrass-invaded areas of the Great Basin (Bradley *et al*., 2006; Prater *et al*., 2006). There are several possible reasons for this discrepancy. First, lower annual precipitation in the Great Basin compared with CSS may limit aboveground NPP of grasses, thus limiting storage in litter and the potential to alter soil storage. Studies in the Great Basin have found evidence of increased C storage in soils of invaded landscapes (Bradley *et al*., 2006; Prater *et al*., 2006), but did not definitively link it to grass invasion. Finally, in contrast to Bradley *et al*. (2006), we worked in invaded landscapes that had not type-converted to grasslands and did not have the associated very recent and repetitive fire history. Manipulative studies to assess mechanisms of C and N storage due to grass invasion vs. loss due to fire are needed in many invaded habitats, including CSS. While CSS can type-convert if fires become very frequent, shrub cover has thus far tended to persist in most invaded areas (Keeley, 2006), as in our study area.

Additionally, the variation in precipitation across the 3 years our study encompassed may have influenced our findings. Our study began in a year (2005) of 190% (521 mm) of the 50-years mean rainfall, resulting in high non-native litter production, and was followed by two moderately dry years (74% and 59% of mean rainfall). Our results may have shown smaller variation in C and N pools if the study had been conducted in average rainfall years. However, CSS is a system punctuated by irregular high precipitation years, often due to ENSO events, and characterized by relatively few average years (Morrison & Bolger, 2002). Importantly, larger fluctuations between high precipitation and drought years are expected in California under most climate change models (Field *et al*., 2007; Kerr, 2008). Thus, we believe our results have important implications for future C balance under a changing climate.

Our findings have large-scale implications for assessing important feedbacks in invasion biology. If plants invade or grow better in high resource areas (Elton, 1958), their tendency to further increase soil resources would promote further invasion (Ehrenfeld, 2003). According to our study, such feedbacks may operate on very short timescales (2 years). Further, because we found invasive plants can rapidly alter soil nutrients, using observational studies to identify pre- vs. postinvasion nutrient patterns may be difficult. Manipulative field experiments, even short-term ones, may help document and quantify postinvasion changes, and should be used more often to study impacts of invasions.

We have highlighted that the interaction between environment and plant functional type and the role of litter are key to understanding mechanisms of C and N storage in plant invasions, and need to be considered when plant invasions lead to domination of a different functional type. Such invasions and the overall expansion of grasses are expected to increase with climate change (Pan *et al*., 1998; Cornwell *et al*., 2008). Therefore, the role of grasses and shrubs in determining C storage in the $\sim$40% of land surface covered by semiarid and arid regions (Throop & Archer, 2008) is important to global models of future C balance.

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Supporting Information

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

Table S1. Estimates of total dry litter (g) added per $9\,m^2$ (per plot) to addition treatment plots, to species. We performed the initial, large litter manipulation after the growing season ended in June 2005, and repeated it in late May–June 2006, though lower precipitation that year led to lower amounts of litter manipulated. In 2005 to bring pre-manipulation plots of low and high invasion levels to the same amount of litter (twice the naturally high abundance) we added twice as much litter to low invasion areas as to high invasion areas.

Table S2. One-way ANOVA results for comparisons of High Invasion × Control to High Invasion × Removal Control show that effects of removal were not due to artifacts (trampling etc.) of manipulation. Litter N results are for Treatment main effect of a GLM that also included 'months in field.'

Table S3. Mean (± SE when available) values of $g\,C\,m^{-2}$ aboveground, in soil and respired in other grassland and shrubland systems. For the cheatgrass invasion we converted soil %C values using a bulk density of 1.5 obtained for nearby study site (Jungo, NV, (United States Department of Agriculture: Natural Resource Conservation Service 2008)).

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