

# Supporting Information

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## SI Methods

**Soil N Analyses.** To determine total inorganic N (TIN) concentrations,  $\approx 10$  g of fresh weight soil per replicate was extracted with 40 mL of 2M KCl, and solutions were filtered through Whatman no. 1 filter paper. Filtrates were then analyzed for TIN ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$ ) with a Lachat autoanalyzer. Microbial biomass N was determined using a simultaneous chloroform fumigation extraction method. Briefly, soil samples were split into 2 subsamples of  $\approx 5$  g of fresh weight soil each. One subsample was extracted with 40 mL of 0.5 M  $\text{K}_2\text{SO}_4$ , and the other subsample was extracted with 0.5 M  $\text{K}_2\text{SO}_4$  + 0.5 mL of chloroform (Acros Organics; catalog no. 61028-1000). Subsamples were then placed on a planary shaker for 4 h at room temperature. Soil suspensions were then filtered through Whatman GF/A filter paper, and filtrates were analyzed for DOC and DON with a Shimadzu TOC analyzer equipped with a DON module. Microbial biomass N values were not corrected for extraction efficiency, and therefore represent extractable rather than total microbial biomass N.

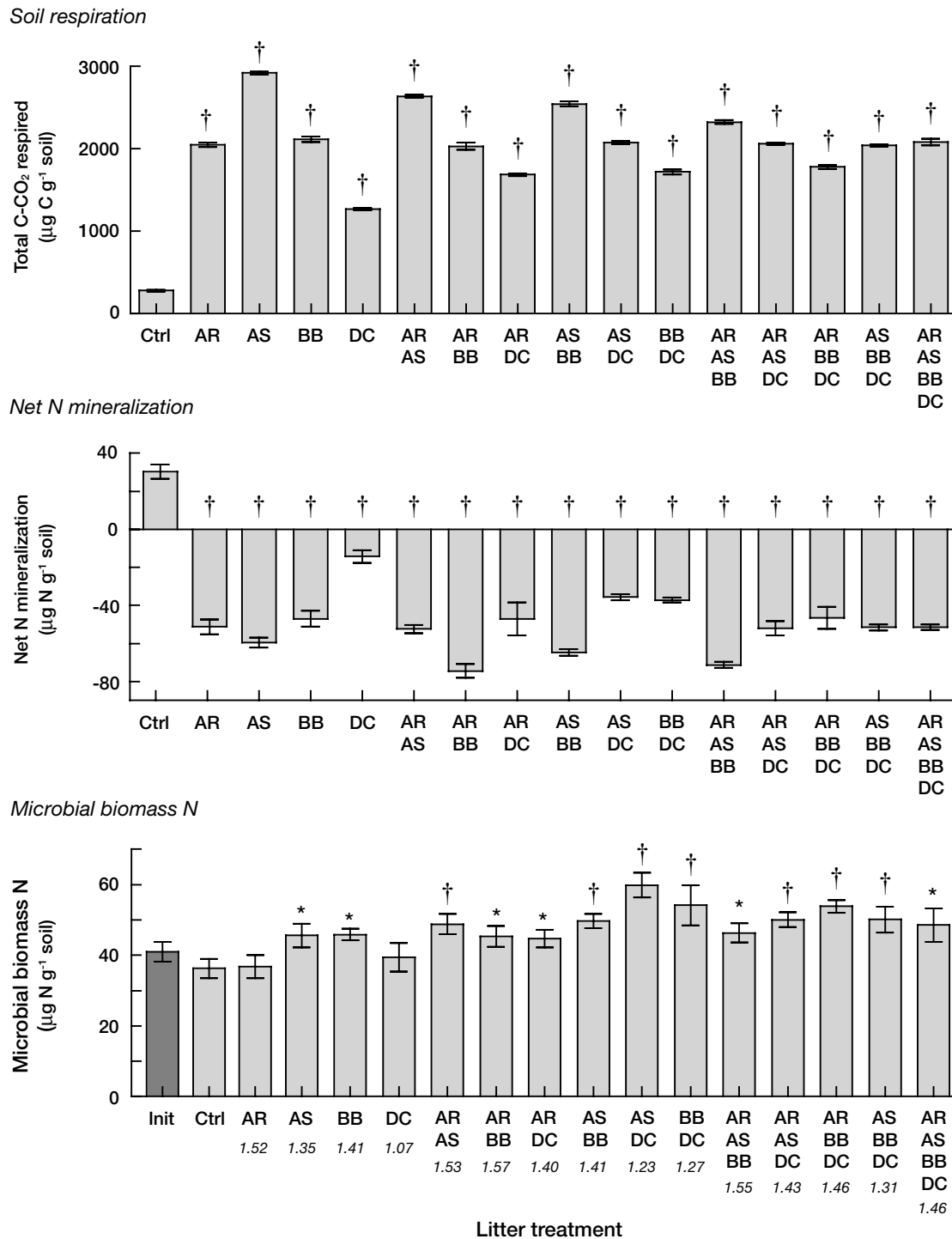
**Condensed Tannin Determination.** To quantify extractable condensed tannins (CT), freeze-dried plant extract was redissolved in 50% aqueous methanol. A 1-mL aliquot of the 50% aqueous methanol solution ( $n = 5$  per species) was then analyzed using the acid-butanol assay. To test for cell-wall-bound nonextractable CT, the acid-butanol reagents were mixed directly with 25 mg of plant litter in  $13 \times 125$  mm glass tubes. Tubes were placed in a boiling water bath for 50 min, and were inverted every 10 min to ensure thorough mixing of the litter with the reagents. Aliquots were then centrifuged ( $10,000 \times g$  for 10 min), and the absorption at 550 nm was read with a spectrophotometer. For both protocols, we used a CT standard purified from *B. bistortoides* litter with Sephadex LH-20 (Amersham Pharmacia). The standard was stored as a freeze-dried powder at  $-20^\circ\text{C}$ , and was dissolved in 50% aqueous methanol immediately before use. We detected no significant difference in *B. bistortoides* litter CT concentrations using the “extractable” and “cell-wall-bound” protocols.

**Proximate C Fractionation.** To assess proximate C fractions for each plant species, 1 g of plant tissue (recorded to 0.1 mg) was extracted with  $2 \times 25$  mL 70% aqueous acetone for 30 min per extraction on a planary shaker. The pellet was then extracted with  $2 \times 25$  mL 50% aqueous methanol for 30 min per extraction.

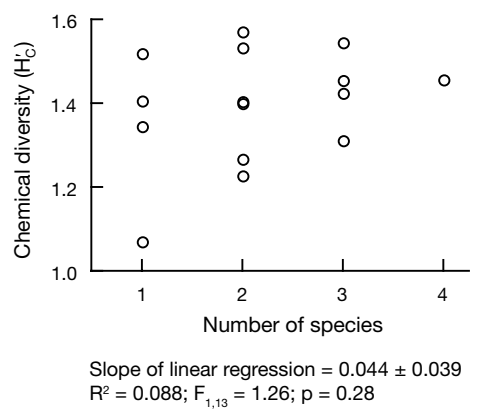
The pellet was then freeze-dried and weighed to the nearest 0.1 mg. The organic soluble fraction (OSF) was calculated as the difference between the organic-extracted pellet mass and the initial litter mass, minus any previously identified organic soluble constituents (i.e., N, CT, HT, sugars, LMW phenolic acids, and other LMW phenolics). The OSF fraction reported in Table S1 is thus residual unidentified organic soluble material. For each species, the freeze-dried organic-extracted pellets were then analyzed for standard acid soluble fractions (ASF) and acid insoluble residue (AIR).

**Characterizing Litter Chemical Composition and Chemical Diversity.** For all analyses, derivatives of 4-hydroxybenzoic acid, caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, and gallic acid were grouped together as low-molecular-weight phenolic acids (Table S1). Similarly, derivatives of anthocyanins, coumarins, and flavonoid glycosides were grouped together as other low-molecular-weight phenolics; and myo-inositol, glucose, fructose, sucrose, and raffinose were grouped together as “sugars” (Table S1). Modeling soil C and N dynamics with grouped versus un-grouped litter chemistry traits revealed that grouping compounds in this way did not affect the statistical outcome of the experiments. “Grouped” chemical traits were therefore used for all analyses to simplify interpretation of the results.

Chemical trait data were centered before performing the PC analysis, and we modeled the soil C and N response to the litter amendments using PC scores generated with the variance scaled to 1 for each chemical trait, or left unscaled. Because the variance of any given chemical trait tends to increase as the mean value of the trait increases, scaling the variance to 1 for each chemical trait before performing the PC analysis gives more emphasis to chemical traits with relatively low concentrations. Comparing “scaled” versus “unscaled” chemical traits thus allowed a quantitative assessment of the importance of chemical traits with low concentration to soil C and N dynamics. For the soil respiration and net N mineralization responses, PC scores derived from scaled and unscaled chemical trait data explained similar amounts of variation (data not shown). In contrast, more variation in microbial biomass N was predicted with PC scores derived from scaled chemical trait data ( $R^2 = 0.29$  using scaled traits,  $R^2 = 0.06$  using unscaled traits). This result indicates microbial biomass N is sensitive to chemical traits with low concentration. We therefore used PC scores derived from scaled chemical trait data to determine the effects of litter chemical composition on all measures of soil C and N cycling.



**Fig. S1.** Soil respiration, net nitrogen mineralization, and microbial biomass N responses to litter treatments. AR, *Acomastylis rossii*; AS, *Artemisia scopulorum*; BB, *Bistorta bistortoides*; and DC, *Deschampsia caespitosa*. In all litter mixtures, component species were of equal abundance by mass. All means are  $\pm 1$  SE ( $n = 8$  per treatment). Asterisks indicate litter amended soils are significantly different from unamended control soils (a priori contrasts; \*,  $P < 0.05$ , †,  $P < \text{Bonferroni-corrected } \alpha$ ). Numbers in italics indicate the chemical diversity ( $H_c$ ) of specific litter amendments, calculated using 9 litter chemical traits known to influence decomposition (traits shown in Fig. 1 and Table S1). See *Materials and Methods* for a description of how  $H_c$  was calculated.



**Fig. S2.** Chemical diversity ( $H_c$ ) of single species and multispecies litter amendments as a function of plant species richness using 4 alpine plant species native to Niwot Ridge, CO.

**Table S1. Litter chemistry traits of the 4 alpine species used to create chemically diverse litter treatments**

Chemistry trait	Abbreviation	<i>A. rossii</i>	<i>A. scopulorum</i>	<i>B. bistortoides</i>	<i>D. caespitosa</i>
Nitrogen	n	0.671 (0.011)	1.74 (0.02)	1.18 (0.03)	0.846 (0.025)
Condensed tannin	ct	0.606 (0.039)	0.162 (0.007)	5.79 (0.20)	ND
Hydrolyzable tannin	ht	23.89 (0.43)	ND	ND	ND
Sugars*	sug	2.27 (0.08)	5.05 (0.07)	3.83 (0.13)	2.61 (0.09)
Low-molecular-weight phenolic acids <sup>†</sup>	phen.a	0.952 (0.045)	1.89 (0.01)	0.166 (0.007)	0.035 (0.001)
Other low-molecular-weight phenolics <sup>‡</sup>	phen.o	2.28 (0.03)	0.594 (0.007)	1.50 (0.04)	0.190 (0.003)
Organic soluble fraction <sup>§</sup>	osf	16.08 (0.63)	26.73 (0.41)	17.53 (0.36)	17.18 (0.25)
Acid soluble fraction	asf	42.99 (0.32)	47.69 (0.24)	48.07 (0.59)	61.1 (1.0)
Acid insoluble residue	aur	11.16 (0.06)	18.20 (0.07)	23.43 (0.18)	19.23 (0.93)
Chemical diversity index <sup>¶</sup>	H' <sub>c</sub>	1.52	1.35	1.41	1.07

Values are mean percentage dry weight ( $\pm 1$  SE). ND, not detected. Litter chemistry traits were assessed using sub-samples ( $n = 5$ ) of bulked litter from each species, collected in late September 2004.

\*The sum of myo-inositol, glucose, fructose, sucrose and raffinose concentrations.

<sup>†</sup>The sum of 4-hydroxybenzoic acid, caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, and gallic acid concentrations. Note that not all LMW phenolic acids were found in all species.

<sup>‡</sup>The sum of anthocyanins, catechins, coumarins, and flavonoid glycosides. Note that not all "other" LMW phenolics were found in all species.

<sup>§</sup>Residual unidentified organic soluble material (identified organic soluble constituents have been subtracted out).

<sup>¶</sup>The chemical diversity index was calculated with the equation for Shannon–Wiener diversity, using the presence and relative abundance of chemistry traits.

**Table S2. PCA axis scores for the 4 species used to create chemically diverse litter treatments**

Plant species	Abbreviation	PC1	PC2	PC3
<i>Acomastylis rossii</i>	AR	-2.89 (0.10)	-1.57 (0.09)	-0.07 (0.02)
<i>Artemisia scopulorum</i>	AS	2.71 (0.04)	-1.68 (0.05)	-0.44 (0.02)
<i>Bistorta bistortoides</i>	BB	0.34 (0.07)	1.17 (0.07)	2.05 (0.13)
<i>Deschampsia caespitosa</i>	DC	-0.16 (0.06)	2.07 (0.01)	-1.54 (0.13)

Values are means  $\pm$  1 SE (in parentheses) for litter samples from each species ( $n = 5$ ) collected in late September 2004.

**Table S3. Regression analyses of soil C and N responses as a function of the abundance of individual species litter within litter mixtures**

Response variable	Plant species	Slope (SE)	$R^2$	$F$	$P$
Total CO <sub>2</sub> respired	<i>A. rossii</i>	-16 (130)	0.00	0.02	0.90
	<i>A. scopulorum</i>	867 (29)	0.34	923.8	<0.0001
	<i>B. bistortoides</i>	-10 (130)	0.00	0.01	0.94
	<i>D. caespitosa</i>	-825 (29)	0.62	1702	<0.0001
Net N mineralization	<i>A. rossii</i>	-14.0 (5.2)	0.057	7.1	<0.01
	<i>A. scopulorum</i>	-16.5 (5.2)	0.079	10.1	<0.01
	<i>B. bistortoides</i>	-14.4 (5.2)	0.060	7.5	<0.01
	<i>D. caespitosa</i>	44.8 (3.5)	0.59	166.7	<0.0001
Microbial biomass N	<i>A. rossii</i>	-9.4 (3.2)	0.066	8.3	<0.01
	<i>A. scopulorum</i>	4.9 (3.3)	0.02	2.1	0.15
	<i>B. bistortoides</i>	2.3 (3.4)	0.00	0.47	0.49
	<i>D. caespitosa</i>	2.2 (3.4)	0.00	0.42	0.52

Simple linear regressions with untransformed response variables were used for all regression analyses.