

Appendices

Appendix A: Illustration of experimental design

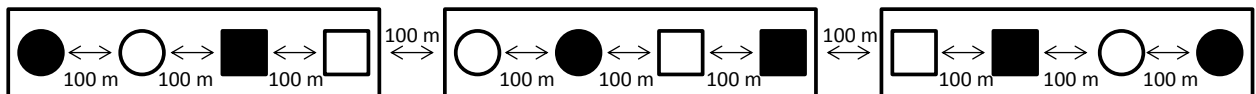
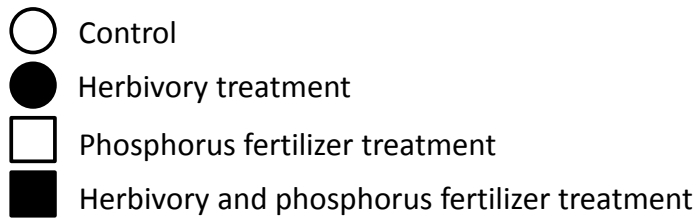
Appendix B: Modified protocol for measuring phosphate

Appendix C: Effect of herbivory and fertilizer treatments on leaf traits

Appendix D: Treatments and covariates affecting aquatic decomposition rates in streams

Appendix E: Treatments and covariates affecting terrestrial decomposition rates in soil

Appendix A. Illustration of experimental design



Appendix B. Modified protocol for measuring phosphate

We boiled down 50mg of leaf powder with 0.4mL 20% $\text{Mg}(\text{NO}_3)_2$ w/v in 95% EtOH and 10mL 1.0M HCL on a 120°C hot plate to 50% of the starting volume. We allowed samples to dry for 48 hours, covered vials with pin pricked aluminum foil caps, and ashed at 550°C in a muffle furnace for 24 hours. Ash was dissolved in 10mL of 0.1M HCL and heated in an 80°C water bath for 30 minutes. We mixed 10 μ L aliquots of sample with 990 μ L DI water and 100 μ L of mixed reagent (containing 5mL 24nM ammonium molybdate, 12.5mL 5N sulfuric acid, 5.0mL 0.31M ascorbic acid and 2.5mL 2.0mM antimony potassium tartrate) in cuvettes to measure total phosphate on a Shimadzu UV-1800 spectrophotometer at 883nm. Reference phosphate compounds returned high yields: 98.6% \pm 0.78SE of 10 μ M glycerophosphate, 102.8% \pm 1.33SE of 10 μ M pyrophosphate, and 93.9% \pm 0.50SE of 10 μ M 5'adenosine monophosphate. We chose 50mg of leaf material per sample after trials of 10, 25, 50 and 100mg. We ran each batch of samples against a five point standard curve of KH_2PO_4 from 0 – 10.0 μ M. To control for across batch variation, we ran controls of 10 μ M 5'adenosine monophosphate, which was chosen because it was the most difficult reference compound to obtain high yields. All batches yielded 90.5 - 99.8% of the expectation for 10 μ M 5'adenosine monophosphate, and correcting for these slight differences did not change conclusions.

Appendix C. Effect of herbivory and fertilizer treatments on leaf traits

Table C1. Percent nitrogen of red alder leaves before an herbivory and fertilizer treatment.

Source	df	SS	MS	<i>F</i>	P (<i>F</i> = 1)
Herbivory treatment	1	0.183	0.183	1.110	0.297
Fertilizer treatment	1	0.438	0.438	2.650	0.109
Herbivory x Fertilizer	1	0.327	0.327	1.976	0.165
Block	20	4.238	0.212	1.282	0.229
Error	57	9.424	0.165		

Table C2. Change in percent phosphorus of red alder leaves following an herbivory and fertilizer treatment.

Source	df	SS	MS	<i>F</i>	P (<i>F</i> = 1)
Herbivory treatment	1	11	11.1	0.060	0.807
Fertilizer treatment	1	680	680.2	3.698	0.0594
Herbivory x Fertilizer	1	15	14.7	0.080	0.7784
Block	20	7400	370.0	2.011	0.0203
Error	58	10669	183.9		

Table C3. Change in percent carbon of red alder leaves following an herbivory and fertilizer treatment.

Source	df	SS	MS	<i>F</i>	P (<i>F</i> = 1)
Herbivory treatment	1	126	125.77	1.055	0.310
Fertilizer treatment	1	33	32.58	0.273	0.604
Herbivory x Fertilizer	1	103	102.68	0.861	0.358
Block	17	2732	160.72	1.348	0.205
Error	48	5723	119.22		

Table C4. Effects of herbivory and fertilizer treatments on several leaf trait variables via multiple analysis of variance

Source	df	Wilk's λ		<i>F</i>	P (<i>F</i> = 1)
		SS	MS		
Herbivory treatment	1	0.529		3.661	0.00235
Leaf Thickness			4.460 ⁻⁶	4.460 ⁻⁶	2.0367
% Nitrogen final			2.256	2.256	8.290
% Carbon final			18.58	18.581	0.710
% Phosphorus final			8.8 ⁻⁵	8.8 ⁻⁵	0.115
Carbon: Nitrogen final			229.13	229.125	9.739
Carbon: Phosphorus final			1	0.6	0.0002
Nitrogen: Phosphorus final			97.77	97.767	13.414

$^{13}\text{C}/^{12}\text{C}$			1.939	1.939	1.707	0.198
$^{15}\text{N}/^{14}\text{N}$			0.0096	0.00956	0.102	0.751
Fertilizer treatment	1	0.863			0.651	0.746
Leaf Thickness			9.167^{-6}	9.167^{-6}	4.187	0.0466
% Nitrogen final			0.0496	0.0496	0.182	0.671
% Carbon final			25.97	25.970	0.992	0.325
% Phosphorus final			1.32^{-4}	1.32^{-4}	0.172	0.680
Carbon: Nitrogen final			53.70	53.704	2.283	0.138
Carbon: Phosphorus final			5534	5533.9	1.566	0.217
Nitrogen: Phosphorus final			0.41	0.408	0.0560	0.814
$^{13}\text{C}/^{12}\text{C}$			2.089	2.089	1.839	0.182
$^{15}\text{N}/^{14}\text{N}$			0.0472	0.0472	0.505	0.481
Herbivory x Fertilizer	1	0.937			0.276	0.977
Leaf Thickness			2.210^{-7}	2.215^{-7}	0.101	0.752
% Nitrogen final			0.0072	0.00718	0.0264	0.872
% Carbon final			15.01	15.014	0.574	0.453
% Phosphorus final			7.6^{-5}	7.614^{-5}	0.0993	0.754
Carbon: Nitrogen final			11.89	11.887	0.0505	0.481
Carbon: Phosphorus final			842	842.5	0.238	0.628
Nitrogen: Phosphorus final			0.00	0.00	0.00	0.999
$^{13}\text{C}/^{12}\text{C}$			0.115	0.115	0.102	0.752
$^{15}\text{N}/^{14}\text{N}$			0.0293	0.0293	0.313	0.579
Block	17	0.0238			1.216	0.0763
Leaf Thickness			7.512^{-5}	4.419^{-6}	2.0182	0.0308
% Nitrogen final			5.167	0.304	1.117	0.369
% Carbon final			515.64	30.332	1.159	0.335
% Phosphorus final			0.0262	0.00154	2.0067	0.0319
Carbon: Nitrogen final			904.43	53.202	2.261	0.0149
Carbon: Phosphorus final			130761	7691.8	2.176	0.0192
Nitrogen: Phosphorus final			94.46	5.556	0.762	0.723
$^{13}\text{C}/^{12}\text{C}$			23.098	1.359	1.196	0.306
$^{15}\text{N}/^{14}\text{N}$			1.544	0.0908	0.971	0.505
Error	45					
Leaf Thickness			9.853^{-5}	2.190^{-6}		
% Nitrogen final			12.245	0.272		
% Carbon final			1178.1	26.180		
% Phosphorus final			0.0345	7.667^{-4}		
Carbon: Nitrogen final			1058.65	23.526		
Carbon: Phosphorus final			159041	3534.2		
Nitrogen: Phosphorus final			327.98	7.288		
$^{13}\text{C}/^{12}\text{C}$			51.118	1.136		
$^{15}\text{N}/^{14}\text{N}$			4.209	0.0935		

Appendix D. Treatments and covariates affecting aquatic decomposition rates in streams

Table D1. Effects of an herbivory treatment and fertilizer treatment on eventual decomposition in stream environments.

Source	df	SS	MS	<i>F</i>	<i>P</i> (<i>F</i> = 1)
Herbivory treatment	1	0.410	0.410	8.605	0.00559
Fertilizer treatment	1	0.082	0.0820	1.723	0.197
Herbivory x Fertilizer	1	0.247	0.247	5.179	0.0284
Block	13	5.484	0.422	8.863	<0.001
Deployment Location	4	5.119	1.280	26.888	<0.001
Land Source	9	0.365	0.0405	0.851	0.575
Error	39	1.856	0.0476		

Table D2. Effects of fertilizer and herbivory treatments on eventual decomposition in stream environments after adjusting for river location and C:N. Testing the herbivory treatment after removing nutrient differences indicates the herbivory treatment was largely a result of nutrient changes, and after removing effects of C:N, the phosphorus fertilizer treatment was significant.

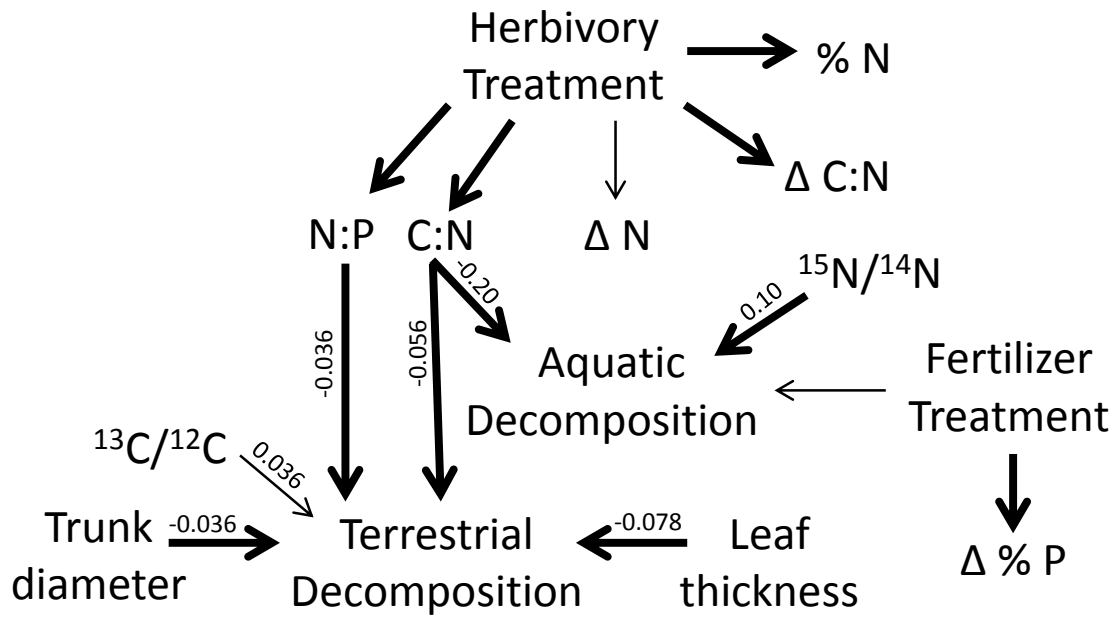
Source	df	SS	MS	<i>F</i>	<i>P</i> (<i>F</i> = 1)
Herbivory Treatment	1	0.0410	0.0410	1.245	0.272
Fertilizer Treatment	1	0.143	0.143	4.329	0.0443
Herbivory x Fertilizer	1	0.124	0.124	3.769	0.0597
Carbon: Nitrogen	1	1.157	1.157	35.107	<0.001
Block	13	5.484	0.422	12.805	<0.001
Deployment Location	4	5.119	1.280	38.849	<0.001
Land Source	9	0.243	0.0270	0.819	0.602
Error	38	1.252	0.0329		

Table D3. Standardized coefficients from type III stepwise regression ($F_{2,53} = 13.3$, $p < 0.001$) showing the effects of various leaf traits on aquatic decomposition. Variables excluded from the model were tree trunk diameter, nitrogen: phosphorus, carbon: phosphorus, $^{13}\text{C}/^{12}\text{C}$, and leaf thickness.

Source	Estimated	t value	Pr(> t)
Carbon: Nitrogen	-0.203	-4.725	0.000133
$^{15}\text{N}/^{14}\text{N}$	0.100	2.330	0.00711

Fig D1. Illustration of herbivory and fertilizer treatments on leaf traits and covariates influencing aquatic and terrestrial soil decomposition. Thick lines are significant ($p < 0.05$) and thin lines are non-significant variables included in stepwise multiple regressions (Table D3, Table E2). Standardized coefficients are reported for effect size comparisons. Significance values of

herbivory treatment on leaf traits obtained from paired t-tests (thick lines, $p < 0.01$; thin lines, $p < 0.05$). Significance of residual effect of fertilizer treatment on aquatic decomposition obtained from Table C2.



Appendix E. Treatments and covariates affecting terrestrial decomposition rates in soil.

Table E1. Effects of an herbivory treatment, fertilizer treatment and leaf source on eventual decomposition in terrestrial soil environments.

Source	df	SS	MS	<i>F</i>	<i>P</i> (<i>F</i> = 1)
Leaf source	1	0.0607	0.0607	7.406	0.00857
Herbivory treatment	1	0.0056	0.00562	0.686	0.411
Fertilizer treatment	1	0.0020	0.00202	0.246	0.622
Herbivory x Fertilizer	1	0.0089	0.0089	1.089	0.301
Block	20	1.151	0.0575	7.024	<0.001
Error	58	0.475	0.00819		

Table E2. Standardized coefficients from type III stepwise regression ($F_{5,56} = 9.967$, $p < 0.001$), showing the association of various leaf traits on terrestrial soil decomposition. Variables excluded from the model were $^{15}\text{N}/^{14}\text{N}$, carbon: phosphorus.

Source	Estimated	t value	Pr(> t)
Leaf thickness	-0.0779	-4.007	0.000183
Carbon: Nitrogen	-0.0563	-3.123	0.00283
Trunk diameter	-0.03589	-2.395	0.0200
Nitrogen: Phosphorus	-0.0362	-2.035	0.0466
$^{13}\text{C}/^{12}\text{C}$	0.0358	1.862	0.0678