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Abiotic nitrate incorporation in soil: is it real?

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Abstract In acid forest soils nitrate (NO_3^-) from anthropogenic nitrogen deposition is retained at levels beyond what can be explained by known biological mechanisms. A number of researchers have hypothesized that abiotic NO_3^- incorporation into soil organic matter might be responsible for this phenomenon, however studies have been limited to a few temperate forest sites. The goal of this study was to determine if abiotic NO_3^- incorporation is important across a wide range of soil types. We collected 44 soils from a number of different ecosystem types in North and South America and measured the extent of abiotic NO_3^- incorporation. Significant abiotic nitrate incorporation did not occur in any of the soils examined. We show that the apparent abiotic incorporation observed in previous studies is likely the result of iron interference with NO_3^- measurements. Our results suggest that abiotic NO_3^- incorporation is not a likely explanation for the high rates of NO_3^- retention observed in some ecosystems.

Keywords Abiotic nitrate incorporation · Iron · Nitrogen deposition · Nitrogen retention

Abbreviations

DNRA	Dissimilatory nitrate reduction to ammonia
DON	Dissolved organic nitrogen
EDTA	Ethylenediamine tetracetic acid
RPM	Revolutions per minute
SOM	Soil organic matter
UV	Ultraviolet
TDN	Total dissolved nitrogen

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Introduction

In acid forest soils the retention of anthropogenic nitrogen deposition is typically very high. For example, in 65 forest stands across Europe receiving ambient N deposition between 1 and >75 kg N ha^{-1}, retention was around 52% (Dise and Wright 1995). In experimental plots receiving between 8 kg N ha^{-1} and 158 kg N ha^{-1} in the US, the lowest rate of retention was 87% (Magill

et al. 1997). This high degree of retention is surprising, given that there do not appear to be corresponding increases in plant growth, microbial biomass, or soil respiration (Aber et al. 1998).

One process that has been postulated to explain the surprising level of nitrate (NO_3^-) retention in forest ecosystems is the abiotic incorporation of NO_3^- into soil organic matter (SOM) (Berntson and Aber 2000). The evidence suggesting that abiotic NO_3^- incorporation may be ecologically important stems from field and laboratory experiments that have reported the rapid disappearance of NO_3^- added to non-sterile (Davidson et al. 1991; Berntson and Aber 2000; Dail et al. 2001; Compton and Boone 2002; Magill et al. 2004; Micks et al. 2004; Ruckauf et al. 2004; Venterea et al. 2004; Westbrook and Devito 2004; Templer et al. 2005), and sterile soils (Davidson et al. 1991; Dail et al. 2001). For example, Davidson et al. (1991) reported that 18% of the $^{15}NO_3^-$ added to non-sterile and sterile soils disappeared within 15 min. Likewise, Dail et al. (2001) reported that within 15 min 30, 40, and 60% of added ^{15}N–NO_3^- was incorporated into non-sterilized, γ-irradiation-sterilized, and autoclave-sterilized soil, respectively. They also found that the majority of the incorporated N had apparently entered the dissolved organic nitrogen (DON) pool, with very little NO_3^- being incorporated into the insoluble SOM fraction.

The direct evidence that abiotic NO_3^- incorporation actually occurs in soil has come from only one temperate forest site (Dail et al. 2001), and neither the kinetics nor the mechanism are known. For these reasons, we wanted to determine if abiotic NO_3^- incorporation is common across a range of ecosystem types, and if it is an important component of the soil nitrogen cycle. We collected soils from 44 sites across North and South America, spanning a variety of parent materials, ecosystem types, and vegetation types. We sterilized soils and measured the rates and extent of abiotic NO_3^- incorporation by measuring the disappearance of added NO_3^- from extractable pools.

Materials and methods

Soil collection

Soil samples were collected from 44 distinct sites throughout North and South America during the peak growing season (Fierer and Jackson 2006). We selected sites that were unsaturated for most of the year. Soils were sampled from the top 5 cm of mineral soil from all sites, and samples were composites of several replicate samples collected from within each site. Soils were shipped to the University of California, Santa Barbara for processing. Soils were characterized using the methods described in Appendix A. The collected soils represented a wide range of soil characteristics with pHs ranging from 3.5 to 8.8 and organic carbon concentrations ranging from 0.08% to 18.24%.

Soil preparation and sterilization

Soils were sieved to 4 mm, thoroughly homogenized, and frozen at –20°C immediately upon arrival. For this study, soils were thawed at room temperature, and nine replicates of 4 g field moist soil were weighed into polypropylene centrifuge tubes. Soils were then sterilized by autoclaving. All approaches for sterilizing soils change the sample to some degree (Wolf and Skipper 1994). We chose autoclaving due to its low cost, safety, and efficacy in eliminating viable microorganisms and their exoenzymes. While autoclaving can change concentrations of extractable Fe and DOM (Wolf and Skipper 1994; Dail et al. 2001), changes in properties such as cation exchange capacity, surface area, and pH are fairly insensitive (Wolf and Skipper 1994). Mercuric chloride ($HgCl_2$) has been proposed as a sterilant that does not alter the overall structure of the organic matter as drastically as autoclaving (Wolf and Skipper 1994), and has been used in studies of the abiotic incorporation of NH_4^+ (Johnson et al. 2000; Barrett et al. 2002), and NO_3^- (Fitzhugh et al. 2003b). However, the addition of $HgCl_2$ introduces a readily reducible cation at high

concentrations (the recommended 5% $HgCl_2$ is 0.18 M Hg^{2+}) that may interfere with a proposed mechanism for abiotic NO_3^- incorporation that depends on redox chemistry (Adamson 1952; Hush et al. 1960; Raposo et al. 2000), such as the hypothesized ''ferrous wheel'' of Davidson et al. (2003). To ensure that soils were sterile, we autoclaved each soil three times (0.5 h at 121°C), with 2 days between autoclave cycles to allow remaining spores to germinate (Wolf and Skipper 1994). Sterilization was confirmed by the absence of CO_2 production and by the absence of visible colony formation when soil suspensions were streaked onto dilute nutrient media agar (data not shown).

Abiotic incorporation experiment

All solutions used in this experiment were autoclaved (1 h at 121°C), and allowed to cool to room temperature. Prior to adding the nitrate solutions to the samples, the sample tubes, solution flasks, and serological pipetter were all surface sterilized with UV radiation in a biocontainment hood. All sterile work was done in the hood to minimize the possibility of reintroducing microbes into the sterilized samples.

Nitrate solutions were made with KNO_3 (Baker, ACS grade) and deionized water. Concentrations used were 0, 0.2, 0.4, 0.6, 1, 2, 3, 4, and 5 mg NO_3^- N l^{-1}. Each replicate soil sample received 20 ml of the appropriate NO_3^- solution and was then shaken at 100 rpm on a rotary shaker for 24 h at 20°C. To extract the NO_3^- held on anionic exchange sites, we added 1.74 g K_2SO_4 to give a final concentration of 0.5 M in the solution, and shook for another 2 h. Solutions were then vacuum filtered using glass fiber A/E filters (Pall Sciences, East Hills, USA), and the filtrate was frozen at –20°C until analysis.

Nitrate incorporation was determined by measuring NO_3^- disappearance from the extractable pool. The initial background NO_3^- pool in each soil was evaluated from the 0 mg N l^{-1} loading solution, the loading solution volume, and the water content of the soil at field weight. This was summed with the amount of NO_3^- added in the loading solution to give total nitrate. The slope of the line of NO_3^- observed vs. total NO_3^- was subtracted from one to estimate the fraction of NO_3^- incorporated.

Nutrient analyses

Nitrate analyses were conducted on a Lachat 2300 autoanalyzer (Lachat Instruments, Milwaukee, USA). The method uses a copperized cadmium column to reduce NO_3^- to NO_2^- at pH 7.5 as buffered by an imidazole buffer (Nydahl 1976; Hales et al. 2004). Briefly, the imidazole buffer was made by dissolving 6.8 g imidazole in 900 ml deionized water, adjusting the pH to 7.5 using concentrated HCl, adding 1 ml of 2% Cu w/v using a $CuSO_45H_2O$ salt, and diluting to 1 l (Patton et al. 2002). Previous studies on abiotic NO_3^- incorporation have used an NH_4Cl/EDTA method to measure NO_3^- concentrations (Quick-Chem-Method-10-107-04-1-A 1995). This method is prone to interference from iron (Vaughan et al. 1993) and could therefore exaggerate the apparent extent of abiotic NO_3^- incorporation (see below). We used an imidazole buffer because we found it to be less sensitive to iron concentration than the NH_4Cl/EDTA method.

Iron interference quantification

To examine the sensitivity of the NH_4Cl/EDTA method to iron interference we made standard additions of either Fe^{2+} ($FeCl_2$, 1,000 ppm in 2% HCl, High Purity Standards) or Fe^{3+} (EDTA ferric sodium salt trihydrate, Acros Organics) to solutions of 4.5 ppm NO_3^- in deionized water. We used concentrations of iron of 0, 50, 100, and 200 mg Fe l^{-1}.

Iron concentration in solutions

Iron concentrations were determined for 0.5 M K_2SO_4 solutions from abiotic incorporation experiments on autoclave sterilized soils by running samples on an AA400 Atomic Absorption Spectrometer (Varian Instruments, Palo Alto, CA, USA) using flame atomization with an air/acetylene flame to vaporize the samples, and values are reported in mg Fe l^{-1}.

Results and discussion

Although the 44 soils included in this study represented a wide range in soil and site characteristics (Appendix A), none of the soils showed any evidence of measurable abiotic NO_3^- incorporation (Fig. 1). Had there been abiotic incorporation, we would have seen the slope of NO_3^- observed versus total NO_3^- diverge from one, or the relationship would have been non-linear. However, the regressions of the NO_3^- observed versus total NO_3^- yielded linear relationships, slopes of one, *y* intercepts equivalent to the zero concentration loading solution, and R^2 values >0.95 in all cases. The highest measured incorporation was 4%, which is indistinguishable from zero and within our range of analytical uncertainty.

While we have demonstrated a lack of abiotic incorporation in mineral soils, Dail et al. (2001) reported abiotic NO_3^- incorporation in an organic soil. Although several of the soils we worked with had high organic matter contents (up to 18%), we also performed our incorporation experiment on an organic soil sent to us by Bryan Dail, from the Harvard Forest site where the Dail et al. (2001) work was performed. We observed 0% incorporation when using the imidazole buffer.

We observed no apparent abiotic NO_3^- incorporation in any of the soils we tested, including the one where it had been previously reported. This finding stands in marked contrast to the results of Dail et al. (2001), who have argued that abiotic NO_3^- incorporation is ecologically important in some soils. A possible explanation for this discrepancy is that the analytical chemistry Dail et al. (and many other researchers) used for determining NO_3^- concentrations is highly sensitive to iron interference, and this iron interference can produce the appearance of abiotic NO_3^- incorporation. The analytical chemistry differs from what we used only in the buffer: previous studies used an NH_4Cl/EDTA buffer; we used an imidazole buffer. We observed significant iron interference at a range of iron concentrations when using the NH_4Cl/EDTA buffer (Fig. 2). With the imidazole buffer, the analysis of NO_3^- concentrations was completely insensitive to iron concentrations. While the NH_4Cl/EDTA buffer method suggests dealing with suspected iron interference by adding EDTA in concentrations above and beyond 1 g l^{-1} (QuickChem-Method-10-107-04-1-A 1995), we observed no decrease in iron interference at any iron concentration (data not shown) with EDTA at concentrations of up to 10 g l^{-1}.

Iron interference would lead one to underestimate NO_3^- concentrations and conclude that there was abiotic incorporation, when in fact none had occurred. This is even the case when using a ^{15}N tracer to assess incorporation as was done by

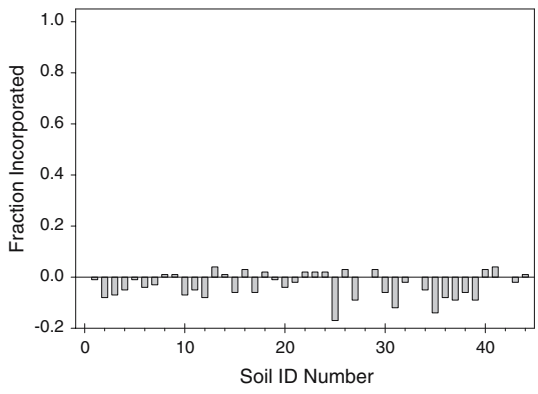

Fig. 1 Fraction of added nitrate incorporated into 44 mineral soils. Soils are identified in Appendix A along with the relevant soil and site characteristics

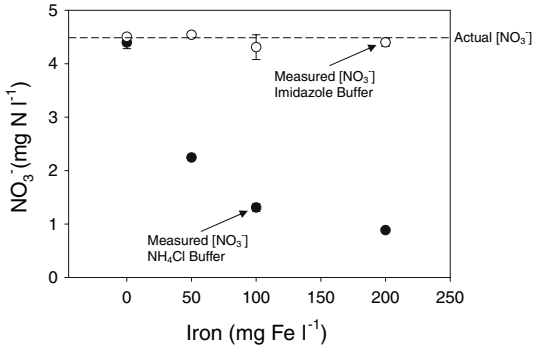

Fig. 2 Measured nitrate concentration of 4.5 mg N l^{-1} NO_3^- solution with added Fe^{2+} iron (results were identical with Fe^{3+} iron). Open circles (○) represent samples run with imidazole buffer, and closed circles (●) represent samples run with NH_4Cl/EDTA buffer. For each data point, $n = 3$; error bars represent the standard deviation of the mean. If error bars are not visible, they are smaller than the data point

Dail et al. (2001), because using ^{15}N to assess incorporation into DON requires both ^{15}N enrichment and accurate pool size numbers to evaluate the fate of ^{15}N-NO_3^-. Evaluating the ^{15}N incorporated into DON is calculated as: ^{15}N-DON = ^{15}N-TDN – ^{15}N-NO_3^- – ^{15}N-NH_4^+, where TDN is total dissolved N. Total dissolved nitrogen is analyzed by alkaline persulfate oxidation (D'Elia et al. 1976) which converts NH_4^+ and DON to NO_3^- and removes iron interference by removing it from solution as an iron oxide precipitate (data not shown). Thus if the NO_3^- pool is underestimated, then not only is ^{15}N-NO_3^- underestimated, but DON, ^{15}N-DON, and apparent incorporation of NO_3^- into DON are all over-estimated. In other words, the ^{15}N would appear to be in the DON pool, when it was actually still in NO_3^-. Dail et al. (2001) used the NH_4Cl/EDTA method for analyzing NO_3^- (Bryan Dail, personal communication). It is therefore highly likely that iron interference resulted in an underestimation of the NO_3^- pool size and an overestimation of NO_3^- incorporation into DON in that work.

To determine if iron interference could actually cause a noticeable overestimate of NO_3^- incorporation in our study, we analyzed the sample solutions from this study with the NH_4Cl/EDTA method. Using this method, we found that apparent incorporation varied from zero to 100% of NO_3^- added, including an apparent incorporation of 44% in the Harvard Forest organic soil where abiotic incorporation had been previously reported (Dail et al. 2001). Not only did the NH_4Cl/EDTA method yield high extents of apparent incorporation in mineral soils (Fig. 3) and the Harvard Forest organic soil, but the apparent incorporation was strongly correlated with iron concentration (R^2 = 0.78), and showed a strong negative correlation with pH (R^2 = 0.45). However, since the apparent NO_3^- incorporation is due to an analytical artifact, this apparent evidence of abiotic NO_3^- incorporation would be erroneous.

Our results indicate that abiotic NO_3^- incorporation is likely negligible in soil and not an important mechanism of N retention in ecosystems. If NO_3^- is not assimilated directly into SOM, we are still faced with the observations that NO_3^- appears to be retained in many soils in apparent excess of biological demand (Aber et al. 1998). One possible explanation for this phenomenon is that the N retention in soil is entirely biological but following assimilation pathways that we do not understand, as suggested by Aber et al. (1998). Another possibility is that nitrite, not nitrate, is abiotically incorporated into soil (Smith and Chalk 1980; Dail et al. 2001; Fitzhugh et al. 2003a, 2003b). Nitrite is an intermediate product of many important biological processes in soil including: denitrification (Firestone 1982), dissimilatory nitrate reduction to ammonia (DNRA) (Silver et al. 2001), and ammonia oxidation (Schmidt 1982). Nitrite is extremely reactive in acid forest soils (Dail et al. 2001; Fitzhugh et al. 2003a, 2003b), unequivocally reacting with organic matter abiotically (Smith and Chalk 1980; Thorn and Mikita 2000).

The finding of iron interference in nitrate concentration measurements has implications beyond research on abiotic NO_3^- incorporation. While we do not know if iron interference has caused widespread errors in assessing soil NO_3^- concentrations, it seems likely that measures of NO_3^- in acid, iron-rich soils may suffer from this artifact, thus underestimating the importance of NO_3^- and overestimating the importance of DON. In addition, estimates of net and gross nitrification rates may in some cases also suffer from an artifact in estimating NO_3^- concentrations.

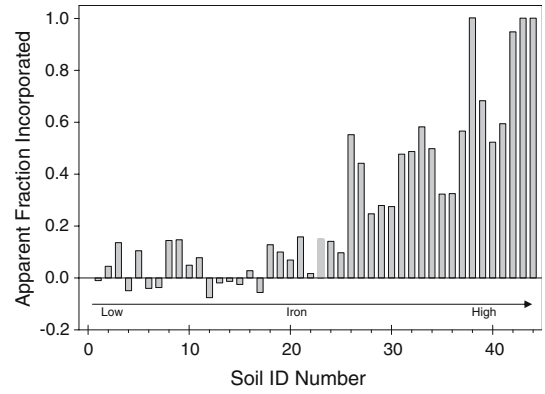

Fig. 3 Apparent fraction incorporated in 44 mineral soils when run with the NH_4Cl/EDTA buffer. Soils are identified in Appendix A along with the relevant soil and site characteristics

Here we have shown that abiotic NO_3^- incorporation does not appear to occur in surface soils, and we suggest that previous reports of abiotic incorporation are likely due to analytical artifacts associated with dissolved iron. Since abiotic NO_3^- incorporation does not appear to occur, we are forced to revisit alternative mechanisms to explain the high rates of N retention observed in many ecosystems.

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Appendix A

Site information and physiochemical properties of the soils used in this study. MAT is mean annual temperature, MAP is mean annual precipitation. All longitudes are West and all latitudes are North with the exception of sites in Peru which are South. The vegetation type at each site was determined in a qualitative manner at the time of sample collection as being coniferous forest, deciduous/broadleaf forest, shrubland, or grassland. Soil organic carbon content was measured on a CE Elantech Model NC2100 elemental analyzer (ThermoQuest Italia, Milan, Italy) with combustion at 900°C, and values are reported in g C 100 g^{-1} soil. Soil pH was measured after shaking a soil/water (1:1 w/v) suspension for 30 min. Soil texture analyses were conducted at the Division of Agriculture and Natural Resources Analytical Laboratory, University of California Cooperative Extension (Davis, CA, USA) using particle size analysis of sand, silt and clay in soil suspension by hydrometer. Iron concentrations were determined for 0.5 M K_2SO_4 solutions from abiotic incorporation experiments as explained in the *Materials and methods*, and values are reported in mg Fe l^{-1}.

Soil ID No.	Location	Latitude	Longitude	Elevation (m)	Dominant plant species	MAT (°C)	MAP (mm)	% Organic C	Texture class	pH	Iron
1	Badlands National Park, SD, USA	43.75	102.38	1000	Grassland	6.6	450	3.1	Silt loam	7.53	0.0
2	Cedar Mountain, AZ, USA	36.05	111.77	2003	Shrubland	10.3	400	2.15	Silt loam	8.02	0.0
3	Calhoun Experimental Forest, SC, USA	34.62	81.67	150	Grassland	15.9	1250	1.71	Sandy loam	5.03	0.0
4	Great Basin Experimental Range, UT, USA	39.33	111.45	3750	Grassland	2	400	2.82	Clay loam	6.84	0.0
5	Konza Prarie LTER, KS, USA	39.10	96.60	100	Grassland	12.5	835	4.62	Silt loam	6.5	0.0
6	Mojave Desert, CA, USA	34.90	115.63	970	Shrubland	21	150	0.08	Sandy loam	8.83	0.0
7	USDA Grassland Research Center, Riesel, TX, USA	31.47	96.87	50	Shrubland	18.1	840	3.94	Silty clay loam	7.92	0.0
8	Hawaii, HI, USA	20.08	155.70	200	Grassland	22.8	250	1.14	Loam	6.45	0.9
9	Hawaii, HI, USA	20.08	155.70	700	Grassland	22.8	750	15.88	Sandy loam	6.32	1.0
10	Institute for Ecosystem Studies, NY, USA	41.80	73.75	75	Grassland	8.6	1200	4.07	Sandy loam	5.52	1.0
11	Duke Forest, NC, USA	35.97	79.08	150	Deciduous/broadleaf forest	14.6	1100	1.7	Loamy sand	5.05	1.0
12	Sequoia National Park, CA, USA	36.50	118.70	650	Shrubland	12.7	650	1.68	Sandy loam	6.25	1.0
13	Toolik Lake LTER, AK, USA	68.63	149.58	894	Shrubland	–9.3	400	15.83	Silt loam	6.47	1.0

Appendix A continued

Soil ID No.	Location	Latitude	Longitude	Elevation (m)	Dominant plant species	MAT (°C)	MAP (mm)	% Organic C	Texture class	pH	Iron
14	Sevilleta LTER, NM, USA	34.33	106.73	1480	Grassland	13.5	210	0.23	Loamy sand	8.44	1.0
15	Itasca State Park, MN, USA	47.18	95.17	550	Coniferous forest	3	750	3.91	Loamy sand	5.42	1.0
16	Santa Barbara, CA, USA	34.47	119.80	500	Shrubland	15	550	2.65	Loam	7.92	1.0
17	Eastern Sierra Nevada Mts., CA, USA	36.45	118.17	3000	Shrubland	3.6	600	1.66	Loamy sand	5.74	1.0
18	HJ Andrews LTER, OR, USA	44.22	122.15	700	Deciduous/broadleaf forest	9.4	2000	7.61	Sandy loam	5.36	2.8
19	Institute for Ecosystem Studies, NY, USA	41.80	73.75	75	Grassland	8.6	1200	2.7	Sandy loam	5.27	3.7
20	Hawaii, HI, USA	20.08	155.70	1000	Grassland	22.8	1000	18.24	Loamy sand	6.53	4.5
21	Calhoun Experimental Forest, SC, USA	34.62	81.67	150	Coniferous forest	15.9	1250	1.21	Loamy sand	4.89	4.7
22	Eastern Sierra Nevada Mts., CA, USA	36.45	118.17	3000	Coniferous forest	3.6	600	4.25	Loamy sand	4.95	6.0
23	Bonanza Creek LTER, AK, USA	64.80	148.25	300	Coniferous forest	–2.9	260	3.73	Silt loam	5.36	10.0
24	Bonanza Creek LTER, AK, USA	64.80	148.25	300	Coniferous forest	–2.9	260	3.03	Silt loam	5.12	10.0
25	Sequoia National Park, CA, USA	36.62	118.63	3215	Grassland	3.6	750	8.1	Loamy sand	5.13	14.3
26	Luquillo LTER, Puerto Rico	18.30	65.83	400	Deciduous/broadleaf forest	21.5	3500	4.11	Silty clay loam	5.03	17.8
27	Bonanza Creek LTER, AK, USA	64.80	148.25	300	Coniferous forest	–2.9	260	3.03	Silt loam	5.16	21.0
28	Manu National Park, Peru	13.02	71.58	3250	Deciduous/broadleaf forest	10	2100	14.9	Loam	3.5	23.5
29	Bear Brook Watershed, ME, USA	44.87	68.10	400	Deciduous/broadleaf forest	6.1	1200	5.22	Sandy loam	4.6	33.2
30	Manu National Park, Peru	13.08	71.58	3250	Deciduous/broadleaf forest	10	2100	13.4	Loam	4.1	35.4
31	Luquillo LTER, Puerto Rico	18.30	65.83	700	Deciduous/broadleaf forest	20.5	4500	6.41	Sandy loam	4.67	37.1
32	Luquillo LTER, Puerto Rico	18.30	65.83	1000	Deciduous/broadleaf forest	19.3	5000	13.95	Silt loam	4.89	44.1
33	Hawaii, HI, USA	20.08	155.70	1500	Grassland	22.8	1500	10.82	Loam	4.92	46.4
34	Mary's Peak, OR, USA	49.47	123.53	1300	Grassland	8.8	2200	10.7	Sandy loam	4.56	49.2
35	Manu National Park, Peru	12.65	71.23	440	Deciduous/broadleaf forest	25	4000	3.3	Clay	4.1	62.1
36	Manu National Park, Peru	12.63	71.27	860	Deciduous/broadleaf forest	23	5000	9.4	Clay loam	3.6	78.0
37	Mary's Peak, OR, USA	49.47	123.53	1300	Coniferous forest	8.8	2200	9.87	Sandy loam	4.38	91.2
38	Catskills, NY, USA	42.16	74.26	800	Deciduous/broadleaf forest	5.3	1300	2.56	Loam	3.92	93.3
39	Toolik Lake LTER, AK, USA	68.63	149.58	894	Grassland	–9.3	400	7.02	Loam	4.58	94.0
40	Harvard Forest LTER, MA, USA	42.50	72.17	300	Coniferous forest	7	1100	9.55	Sandy loam	3.98	97.6
41	Toolik Lake LTER, AK, USA	68.63	149.58	894	Shrubland	–9.3	400	5.39	Loam	4.23	101.6

Appendix A continued

Soil ID No.	Location	Latitude	Longitude	Elevation (m)	Dominant plant species	MAT (°C)	MAP (mm)	% Organic C	Texture class	pH	Iron
42	Bear Brook Watershed, ME, USA	44.87	68.10	400	Coniferous forest	6.1	1200	12.84	Sandy loam	4.25	103.2
43	Catskills, NY, USA	41.93	74.35	800	Deciduous/broadleaf forest	5.3	1300	4.06	Sandy loam	3.63	106.2
44	Catskills, NY, USA	42.12	74.10	800	Coniferous forest	5.3	1300	4.33	Silt loam	3.56	216.0

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