Nitrogen dynamics in arctic tundra soils of varying age: differential responses to fertilization and warming

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1   **Abstract**

2   In the northern foothills of the Brooks Range, Alaska, a series of glacial retreats has created a landscape that

3   varies widely in time since deglaciation (= soil age), from ~10k years to more than 2M years. Productivity of

4   the moist tundra that covers most of this landscape is generally N-limited, but varies widely, as do plant-species

5   composition and key soil properties such as pH. These differences might be altered in the future because of the

6   projected increase in N availability under a warmer climate. We hypothesized that future changes in

7   productivity and vegetation composition across soil ages might be mediated through changes in N availability.

8   To test this hypothesis, we compared readily available-N (water-soluble ammonium, nitrate, and amino acids),

9   moderately-available N (soluble proteins), hydrolysable-N, and total-N pools across three tussock-tundra

10   landscapes with soil ages ranging from 11.5k to 300k years. We also compared the effects of long-term

11   fertilization and warming on these N pools for the two younger sites, in order to assess whether the impacts of

12   warming and increased N availability differ by soil age.

13   Readily available N was largest at the oldest site, and amino acids (AA) accounted for 80-89 % of this

14   N. At the youngest site, however, inorganic N constituted the majority (80-97%) of total readily-available N.

15   This variation reflected the large differences in plant functional-group composition and soil chemical properties.

16   Long-term (8-16 years) fertilization increased soluble inorganic N by 20-100 fold at the intermediate-age site,

17   but only by 2-3 fold at the youngest-soil site. Warming caused small and inconsistent changes in the soil C:N

18   ratio and soluble AA, but only in soils beneath *Eriophorum vaginatum*, the dominant tussock-forming sedge.

19   These differential responses suggest that the impacts of warmer climates on these tundra ecosystems are more

20   complex than simply elevated N mineralization, and that the response of the N cycling might differ strongly

21   depending on the ecosystem's soil age, initial soil properties, and plant-community composition.

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24   *Amino acids, Available nitrogen, Hydrolysable nitrogen, Plant community composition,*

25   *Proteins*

26

27   Dissolved organic nitrogen   DON

28   Amino acids   AA

29   Hydrolysable ammonium   HAm

30	Hydrolysable amino acid	HAA	
31	Hydrolysable amino sugars	HAS	
32			

Introduction

34 Plant productivity in arctic-tundra ecosystems is generally N-limited despite the large amount

35 of N stored in their soils (Schimel et al. 1989; Shaver et al. 1992). The N limitation arises

36 because microbial decomposition of soil organic matter is slow under cold temperatures and

37 therefore only a small portion of the stored N becomes available each year; inputs by

38 deposition and fixation are very small (Hobara et al. 2006). A key strategy of plant survival

39 might involve the acquisition of N in different chemical forms (McKane et al. 2002). The

40 currently slow decomposition rates at these latitudes are expected to increase in the future

41 under a warmer climate, leading to an increase in overall N availability. The increase in N

42 availability might be accompanied by changes in the relative availability of different forms of

43 N and their uptake by plants, resulting in changes in plant productivity and species

44 composition (McKane et al. 1997; Shaver et al. 2000).

45 Moist tussock tundra is one of the most common types of tundra in arctic Alaska,

46 USA (Shaver et al. 1991). Although most tussock tundras are N-limited (Shaver and Chapin

47 1995), differences in N availability and plant-species composition occur across tussock

48 tundra landscapes of different age , resulting from different glaciation histories (Gough et al.

49 2000; Hamilton 1978; Hamilton 2003). Differences in N availability might be partly

50 controlled by differences in pH and chemical properties of the soil, because these differences

51 can affect microbial mineralization and transformation (Whittinghill and Hobbie 2011)

52 Variations in soil pH are consistent with tundra age: moist tundra on intermediate-age and old

53 glacier drifts (>50k years old) are more acidic (soil pH 4-5) and have lower available base

54 cations than tundra on young glacier drift (11.5-25k years old, soil pH 6-7) (Hahn et al.

55 1989; Hobbie and Gough 2002; Walker and Walker 1989). Decomposition rates measured as

56 cumulative field respiration of soil C are faster on older sites, and available inorganic N is

57 higher in younger soils (Hobbie and Gough 2004; Hobbie et al. 2002). The available N pool

58 of both intermediate-age and young sites is dominated by ammonium (NH_4^+) and soluble

59 amino acids (AA), whereas nitrate (NO_3^-) is a minor component everywhere (Hobbie and

60 Gough 2002; Nordin et al. 2004). These differences in soil biogeochemistry are associated

61 with differences in plant productivity and species composition among these tundra landscapes

62 (Gough et al. 2000). Long-term field experiments in both intermediate-age and young

63 tussock tundra that mimic the effects of warmer climate by raising nutrient availability (i.e.,

64 through fertilization) and by warming (in a plastic greenhouse) have revealed that these

65 different-aged ecosystems responded differentially to the treatments (Gough and Hobbie

66 2003; Hobbie et al. 2005). Nonetheless, underlying N cycling processes that are likely

67 responsible for the variation in plant-species composition and responses to the experimental

68 treatments across different-aged tundra ecosystems are still poorly understood.

69   Although release of readily-available inorganic N (NH_4^+, NO_3^-) might be linked

70 directly to the internal metabolism of microbes and to turnover of their biomass (i.e.,

71 nitrification and mineralization), the major process responsible for the release of readily-

72 available organic N (i.e., AA) is the proteolysis of soluble proteins and peptides by

73 extracellular enzymes produced by these microbes (Lipson and Näsholm 2001; Schimel and

74 Weintraub 2003). Most of the proteins and peptides are released upon lysis of dead microbial

75 cells. In an alpine tundra ecosystem, Lipson et al. (1999) found that soluble proteins peaked

76 in soil after snowmelt while microbial biomass declined sharply after reaching its maximum

77 under snowpack. Less is understood about dynamics of other organic-N pools that are not

78 soluble, even though they account for the largest fraction of soils (Myrold 1998; Yano et al.

79 2010). These organic-N pools include hydrolysable amino acids (HAA) and amino sugars

80   (HAS). There is some evidence that N becomes available from these pools via gradual

81   decomposition. For example, Zhang et al. (1999) found that the cultivation of native

82   grassland for >80 years reduced hydrolysable amino sugar concentration in the soil by 6%.

83   In a microcosm experiment in which spruce seedlings were grown in a forest (Oa horizon)

84   soil for 145 days, Johnsson et al. (1999) found that hydrolysable amino acids or amino sugars

85   associated with the humin fraction of soil decreased significantly. These results suggest that

86   HAS and HAA pools might serve as a long-term storage for N, which slowly becomes

87   available under increased soil microbial activity and/or a prolonged increase in N demand in

88   the system without additional N inputs. However, little is known about how warmer climate

89   might affect these hydrolysable N pools. Additionally, because almost all HAS in soil

90   originates in peptidoglycans and chitins of microbial cell walls whereas proteins can

91   originate from both plants and microbes (Sterner and Elser 2002), shifts in N availability and

92   soil microbial activity might be reflected in HAS-to-HAA ratios.

93   In this study, we assessed how N cycling differs across different-aged tundra

94   landscapes with distinct plant-species composition by examining partitions of N among

95   readily-available N (extractable NH_4^+, NO_3^-, and AA), moderately-available N (soluble

96   proteins), and hydrolysable-N pools in three tussock-tundra sites. Soil age of these sites

97   ranged from 11.5 to 300k years old. We hypothesized that a greater fraction of N would be

98   found as soluble proteins or hydrolysable-N pools as soil age increases because of prolonged

99   N incorporation into plant biomass and accumulation of organic matter at older sites. This

100   pattern might mean that plant production at older sites relies on greater capability of

101   proteolysis and production of AA relative to younger sites. Additionally, we hypothesized

102   that a warmer climate would affect N dynamics differently across these different-aged tundra

103   ecosystems. To test this hypothesis, we investigated at intermediate-age (50k-120k years)

104   and young (11.5-25k years) sites whether experimentally elevated N availability (achieved

105 directly by artificial fertilization and indirectly by warming) would alter the balance among

106 AA, inorganic N, and hydrolysable-N pools.

Materials and methods

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Study Sites

108

109 The study was conducted in old, intermediate-age, and young moist-tussock tundra

110 ecosystems with different deglaciation history. Soils of the two older sites are acidic because

111 of longer durations of soil development processes at these sites, whereas soils of the young

112 site remain non-acidic (Table 1). All sites are located within 11 km of the Arctic Long Term

113 Ecological Research (LTER) site at Toolik Lake (68°38' N, 149°36' W, 760 m above sea

114 level) on the northern foothills of the Brooks Range, Alaska, USA. Average annual air

115 temperature at the LTER site was -8.5 °C and annual precipitation was 323 mm during 1989-

116 2008. The old site is located on the middle of the east-facing slope along Imnavait Creek, ~1

117 km south of a gauging station on Imnavait Creek and ~11 km east of Toolik Lake. The

118 sample plots were located away from watertracks (vegetation bands running downslope with

119 high shrub density and higher water flow, compared to the surrounding tundra). The soil of

120 this site is approximately 300k years old (Hamilton 1978; Hamilton 2003). The other two

121 sites are located ~500 m from Toolik Lake: the intermediate-age site (50-120k years old) is

122 located on the south side of the lake, and the young site (11.5-25k years old) on the west side.

123 The intermediate-age site was also called moist acidic tundra or MAT, and the young site as

124 moist non-acidic tundra or MNT, elsewhere (Hobbie et al. 2002; Nordin et al. 2004). At all

125 sites, *Eriophrum vaginatum* is responsible for the formation of tussock microtopography, and

126 the depressions between tussocks (inter-tussock) are dominated by mosses and shrubs.

127 Continuous permafrost underlays all sites, and a peaty organic horizon approximately 10-20

128 cm thick occurs beneath the live vegetation (Hobbie et al. 2002; Yano et al. 2010). Soil

129   properties, biomass, and the composition of plant growth forms differed across the sites,

130   generally showing greatest extractable cations at the young site, greatest relative dominance

131   of shrubs at the intermediate-age site, and greatest moss abundance at the old site (Table 1).

132   Four replicate blocks were established on the intermediate-age site in 1989 and three

133   blocks on the young site in 1997. Each block contained randomly-assigned 5 m x 20 m

134   fertilizer, greenhouse, and control plots (one each/block). The fertilizer plots received 10 g N

135   m^{-2} yr^{-1} as NH_4NO_3 and 5 g P m^{-2} yr^{-1} as P_2O_5 or triple superphosphate applied manually as

136   agricultural fertilizer pellets between late May and early June once the ground became snow-

137   free (Shaver et al. 2000). The greenhouses covered an area of 12 m^2 and were built of plastic

138   sheets that were placed over greenhouse frames in early June and removed at the end of the

139   growing season (late August). Air temperature inside the greenhouses during the growing

140   season was raised by 3-5°C, relative to ambient air (Shaver et al. 2000). Details of the

141   fertilizer applications and greenhouse design can be found in Chapin et al. (1995). The total

142   duration of treatment application prior to this study was 16 yrs for the intermediate-age site

143   and 8 yrs for the young site.

144   **Soil sampling and extraction**

145   In late July 2005, three sampling plots (approximately 5 m x 5 m) were selected from the old

146   site, with plots spaced ~50 m apart. Within each plot, three cores each (diameter 5.4 cm)

147   were collected at random locations in tussocks and in inter-tussock spaces. Upon collection,

148   the top 15 cm of soil beneath a surface layer of live moss and aboveground plants was cut out

149   from the core with a knife for further processing. Live roots were immediately removed in

150   the field from the sample by hand and the soil homogenized manually. The three cores of the

151   same soil type (tussock or inter-tussock) were composited by plot prior to analysis (n=3 for

152   tussock or inter-tussock soils). All soil handling was performed with clean Nitrile gloves.

153   At the intermediate-age and young sites, three cores each of tussock and inter-tussock

154   samples were collected from the control and fertilization treatment plots of three treatment

155   blocks. The cores were handled in the same manner as for the old site and combined by

156   treatment block (n=3 per tussock or inter-tussock). For the warming treatments, three cores

157   each (at the intermediate-age site) and two cores each (at the young site) were collected from

158   tussock and inter-tussock within the greenhouse, and each core was considered to be one

159   sample (n=3 for the intermediate-age site and 2 for the young site per tussock or inter-

160   tussock).

161   For all three sites, the samples were placed in clean plastic bags and transported to the

162   Toolik Field Station, where soil samples were extracted and their moisture content

163   determined. In some cases, the soil cores from the intermediate-age and young sites had

164   organo-mineral soil at the bottom of 15-cm cores (Table 1). The organo-mineral soil was not

165   removed, because root distribution did not change abruptly across the divide between this

166   layer and the organic soil above it. Subsamples of homogenized soils were dried at 60°C to

167   determine moisture content and bulk C and N. Dried soil samples were ground to pass a

168   0.15-mm screen and analyzed using a ThermoScientific 2000 CN Analyzer at the Ecosystems

169   Center, MBL, Woods Hole, MA. Two sets of subsamples (20-50g, wet mass) were placed

170   separately in clean air-tight plastic bags and stored frozen for hydrolysis. We chose freezing

171   over drying, because these soils are subject to freezing temperatures even in summer. We

172   assumed that the impact of microbial cell lysis by freezing on hydrolysable pools would be

173   negligible because chloroform-extractable N could account for <1% of total N for soils near

174   the Old site (Yano et al. 2010). Extraction methods used for available and potentially-

175   available N pools (NH_4^+, NO_3^-, AA, and proteins) were modified from that of Lipson et al.

176   (1999) and (Weintraub and Schimel 2005), and all extraction procedures were conducted

177   within 14 hrs of collection. Sixty milliliters of deionized (DI) water were added to 20 g

178　subsamples (wet mass) of each sample and extracted for 30 min at room temperature on a

179　shaker table. The extractants were filtered through ashed GF/F glass-fiber filters, and

180　approximately half of each extractant was diluted with DI water at a 1:1 ratio for analysis of

181　NH_4^+ and NO_3^-, and dissolved organic N (DON). The remaining extractant was kept without

182　dilution for AA analysis. To extract soluble proteins with minimal destruction of microbial

183　cells, another set of subsamples (10 g, wet mass) was mixed with 50 mL of 0.1 M $NaHCO_3$

184　(Ladd and Paul 1973), gently shaken on a shaker table for 1 hr at room temperature, and

185　filtered through ashed GF/D glass fiber filters. All extractants were stored frozen until

186　analysis.

187　**Soil N fractions**

188　We determined the size of available N pools as water-extractable ammonium (NH_4^+), nitrate

189　(NO_3^-), and amino-acid (AA) fractions of total soil N. Moderately-available N was

190　determined as a $NaHCO_3$-soluble protein fraction of total N. We defined hydrolysable N as

191　types of soil N that can slowly become available over the long term: hydrolysable-ammonium

192　(HAm), amino-acid (HAA), and amino-sugar (HAS) pools were determined as fractions of

193　total N. Dissolved organic N (DON) was determined as the difference between total

194　dissolved N (TDN) and dissolved inorganic N (DIN, NH_4^+ plus NO_3^-) in water extracts and

195　reported as a fraction of total N.

196　　　Water-extracted NH_4^+ was determined by the hypochlorite-alkaline phenol method

197　(Weatherburn 1967), NO_3^- was determined by ion chromatography (Dionex, Sunnyvale, CA,

198　USA), and TDN was determined by a high-temperature combustion method (Shimadzu,

199　Columbia, MD, USA). Water-extractable AA concentrations were determined as described

200　in (Lipson and Monson 1998), but without the concentration step. In brief, AA was

201　determined by subtracting NH_4^+ determined by the hypochlorite-alkaline phenol method

202　from total AA plus NH_4^+ determined by the ninhydrin method (Moore and Stein 1954; Rosen

203   1957). Soluble proteins were determined using the Bradford assay (Bradford 1976) with a

204   liquid bovine serum albumin standard (Sigma-Aldrich) as analytical standard. Protein-N

205   fraction was estimated using the average N content for the twenty amino acids (17%) in

206   proteins (Sterner and Elser 2002).

207   Hydrolysable ammonium, HAA, and HAS were determined using the Mulvaney and

208   Khan (2001) method but replacing their titration method with the hypochlorite-alkaline

209   phenol method for NH_4^+ analysis (Yano et al. 2010). Soils were thawed and 5 g of ground

210   samples were hydrolyzed in 6 M HCl for 12 hours. The hydrolysates were neutralized with

211   NaOH, filtered through Whatman #50 filters, and stored at 5°C until further analysis. The

212   hydrolysates were diffused with either MgO (for HAm) or NaOH (for HAm, HAS) in the

213   presence of acid traps (5 mL of 4% H_3BO_3), and NH_4^+ collected in the acid trap was analyzed

214   by the hypochlorite-alkaline phenol method. HAA remaining in the hydrolysate after

215   diffusion for HAm and HAS was converted to NH_4^+ by ninhydrin reaction (Mulvaney and

216   Khan 2001) and re-diffused with NaOH followed by NH_4^+ analysis. Average N recoveries of

217   standard compounds (percent $\pm$ 1 SE) after diffusion (and ninhydrin reaction for amino acids)

218   were: $NH_4^+ = 96.5 \pm 1.8$, glucosamine $= 100.2 \pm 1.57$, and glycine $= 101.3 \pm 2.38$.

219   **Protease activity**

220   Protease activity was measured using a combination of the Lipson et al. (1999) and

221   Weintraub and Schimel (2005) methods used previously for alpine- and arctic-ecosystem

222   soils. Five grams (wet mass) of subsamples from the homogenized soil samples were mixed

223   with 40 mL of sodium-citrate buffer (pH 5.2) and 0.4 mL of toluene that inhibits microbial

224   uptake of amino acids. The mixture was incubated at 5°C and subsamples (2.0 mL) were

225   taken at 5 min (as t=0), 4 h, and 6 h; the reaction was stopped by adding 2.0 mL of TCA

226   solution (0.11 M trichloroacetic acid, 0.22 M sodium acetate, and 0.33 M acetic acid)

227   (Watanabe and Hayano 1995). The subsamples were then frozen until AA analysis occurred,

228   as described above. Protease activity was calculated as an average of the first (0-4 hr) and

229   second (4-6 hr) periods as described in Weintraub and Schimel (2005).

230   **Statistical analysis**

231   To test our hypotheses that availability and partitioning of N differ 1) across soil age, 2)

232   across the fertilization/warming treatments within sites, and 3) between soil types (tussock vs.

233   inter-tussock), we used two-factor factorial analysis of variance (ANOVA), followed by

234   contrasts of treatment effects. In the analysis, each site-treatment combination was regarded

235   as one factor with seven levels (i.e., young site-control; young site-fertilization; young site-

236   warming; intermediate site-control; intermediate site-fertilization; intermediate site-warming;

237   old site-control), and the soil type as the other factor with two levels (i.e., tussock; inter-

238   tussock). Statistical analysis was performed using JMP 10.0.0 (2012 SAS Institute Inc.,

239   Cary, NC, USA). When necessary, data were transformed to obtain equal variances prior to

240   statistical analysis.

241

242 # Results

243   **Site effect on N pools**

244   Soil age had relatively minor influence on total N, but soil C:N ratios were

245   consistently greater in older soils and in tussock compared to inter-tussock soils (Table 1, 2).

246   The higher C:N ratios were mostly because of a tendency toward greater total C in older sites

247   and in tussock versus inter-tussock soils (Table 1). For both tussock and inter-tussock soils,

248   total readily-available N was greatest at the old site, because of the 2-9 times greater AA

249   concentrations in this site relative to the younger sites (Fig. 1a, Table 2). AA comprised a

250   greater proportion of readily-available N with increasing soil age: 80-89% at the old site, 43-

251   67% at the intermediate-age site, and 3-20% at the young site. In contrast, inorganic N (NO_3^-

252    $+ NH_4^+$) accounted for 80-97% of total available N at the young site. The concentrations of

253   inorganic N were 2–7 times greater at the young site than at the two older sites, and NH_4^+

254   accounted for 63-82% of total available N at the young site. The soluble-NO_3^- fraction was

255   generally a minor proportion of total available N and that proportion was similar across soil

256   types (tussock vs. inter-tussock) and soil ages (Fig. 1a).

257   Among-site differences in soluble proteins (moderately-available N) mirrored patterns

258   observed for AA. Soluble protein concentrations differed significantly among sites and

259   between soil types; highest concentrations occurred at the old site, and were progressively

260   lower with decreasing soil age (Fig.1b, Table 2). In contrast with AA and soluble proteins,

261   protease activity did not vary significantly across sites, and variability within sites and soil

262   types was large (Fig. 1c, Table 2).

263   Total hydrolysable-N concentrations were similar among sites and between soil types.

264   The HAA was consistently the largest pool of the three hydrolysable-N pools for all the soils,

265   accounting for roughly 60-70% of the total hydrolysable N (Fig. 2a, Table 2). Site age

266   significantly affected HAS:HAA ratios; the highest ratios occurred at the young site, and

267   were lowest at the intermediate-age site (Fig. 2b, Table 2).

268   **NP Fertilization & warming effects on N pools**

269   Neither the fertilization nor the warming treatment had a significant effect on total N

270   concentrations, but both significantly altered C:N ratios in tussock soils (Fig. 3a, Table 3).

271   Fertilization always lowered the C:N ratio, mainly because of a tendency toward lower total

272   C under this treatment (data not shown). By contrast, warming raised the mean C:N ratio at

273   the intermiedate-age site and lowered the ratio at the young site.

274   Fertilization tended to have a greater effect on readily-available N pools at the

275   intermediate-age site than at the young site and in tussock compared to inter-tussock soils

276   (Fig. 3b, Table 3). Soluble inorganic N was significantly elevated at both fertilized sites. The

277 mean soluble inorganic-N concentrations were elevated by 20 (inter-tussock) to 100 (tussock)

278 times under the fertilization treatment relative to the control at the intermediate-age site. By

279 contrast, inorganic N concentrations were elevated only by 2 (inter-tussock) to 3 (tussock)

280 times at the young site. Soluble AA concentrations were significantly elevated by both

281 fertilization and warming treatments, but only in tussock soils at the intermediate-age site

282 (Fig. 3b, Table 3). The warming treatment generally had little effect on all soluble-N pools,

283 and neither soluble-protein concentration nor protease activity was affected by the

284 fertilization (Fig. 3b-d, Table 3); native protein levels (Fig. 1b) were maintained regardless of

285 the field manipulation.

286   Both fertilization and warming treatments had little effect on total hydrolysable-N

287 pools, except in tussock soils of the young site, where total hydrolysable N under the

288 fertilization treatment was elevated significantly relative to the control (Fig. 4a, Table 3).

289 Although fertilization did not have a consistent effect on total hydrolysable N, the treatment

290 elevated HAS:HAA ratios, in most cases (Fig. 4b, Table 3).   Soil type significantly affected

291 total hydrolysable N at the intermediate-age site, having consistently higher concentrations in

292 the inter-tussock soils than tussock soils (Fig. 3a, Table 3).

293

Discussion

294

Among-site differences

295

296 Our findings provide quantitative assessment of the effects of soil age, fertilization, and

297 experimental warming on a wealth of N compounds at the peak of growing season in arctic

298 tussock tundra ecosystems. Our results are consistent with previous findings by Nordin et al.

299 (2004) who found greater NH_4^+ abundance relative to NO_3^- in inter-tussock soils at the young

300 site compared to the intermediate-age site throughout the growing season (June-August). In

301 spite of different years, dates of sampling, and extraction methods, readily-available N

302 measured for the intermediate-age and young sites in this study and previous studies were

303 comparable (see comparison table in the Appendix). Some differences in the values likely

304 resulted from inter-annual variations in environmental conditions and ecosystem productivity.

305 　　　Partitioning of total N among readily available and moderately available forms

306 differed among the sites, and these partitioning patterns were not related to total N but

307 apparently related to soil properties. At the young site, lower available cation exchange

308 capacity of soils (Table 1) might be partly responsible for the relatively high water-

309 extractable NH_4^+. Hobbie and Gough (2002) found that available cation exchange sites in

310 organic soils at the young site were nearly three times lower than at the intermediate-age site,

311 likely because of the eight-fold higher Ca^{2+} concentration in the former site, even though total

312 cation exchange capacity was greater at the young site. In contrast, more cation exchange

313 sites might be available at the old site, because the site had low Ca^{2+} concentration and nearly

314 twice the moss biomass as the young site (Table 1). Peat derived from *Sphagnum* mosses,

315 one of the most abundant species in our study sites, possesses strong cation exchange

316 capacity that derives from uronic acid, amino acids, and phenolic acid contents of cell walls

317 (Richter and Dainty 1989).

318 　　　The partitioning of available N forms across sites might also result from differences in

319 N demand of vascular plants and their general preference of NH_4^+ over other forms of N.

320 Aboveground biomass of vascular plants was approximately twice as large at the

321 intermediate-age site (308 g/m^2) as at the other two sites (133 for the old site and 194 g/m^2

322 for the young site, Table 1). When taking into account the nearly twice as high annual N

323 demand of graminoids compared to deciduous or evergreen shrubs (Shaver and Chapin

324 1991), N demand of vegetation at the intermediate-age site would be >2 times that of the

325 demand at the old site. N demand by mosses is expected to be minimal, because they can

326 recycle most of their biomass N to new aboveground growth, and the new growth has a

327 multiple-year lifespan (Aerts et al. 1999; Eckstein 2000). Furthermore, while many vascular

328 plants in arctic and boreal ecosystems appear to have the ability to use inorganic N and at

329 least some AA, studies have shown that plants more frequently used NH_4^+ than AA (Näsholm

330 et al. 1998; Nordin et al. 2001; Nordin et al. 2004). Perhaps the low NH_4^+ and AA at the

331 intermediate-site partly explained by high plant N demand and the relatively high NH_4^+

332 accompanied by the high AA at the old site is explained by low plant N demand.

333   Alternatively, the progressively greater concentrations of soluble-AA and protein with

334 increasing soil age might indicate greater proteolysis at older sites. Given the similar protease

335 activity across all sites, the greater proteolysis was in turn driven by the higher availability of

336 substrates at older sites (Fig. 1c, but see discussion below on protein concentrations). This

337 idea is consistent with Lipson et al. (1999), who concluded that low AA concentrations in

338 alpine tundra soils during the middle to late portions of the growing season was caused by

339 substrate limitation, because the low AA concentrations coincided with low soluble proteins

340 while protease-substrate reaction did not appeared to be saturated. In contrast, Weintraub and

341 Schimel (2005) concluded that a decline in AA concentrations in arctic tussock-tundra soils

342 during the growing season reflected strong N demand by plants and the microbial community

343 associated with them, because protease-substrate reactions were not saturated during most of

344 the growing season. These variable results probably reflect complex biogeochemical

345 processes that work simultaneously to control availability of substrates, products of enzyme

346 reaction, and biological N demand. For example, the high abundance of *Sphagnum* mosses

347 and peat at the old site might have contributed to the large soluble protein pool, given that

348 mosses can retain dissolved proteins via cation-exchange and a reaction with keto-carboxylic

349 acid groups in their cell walls (Painter 1983; Painter 2003). However, these protein-binding

350 reactions might also hinder proteolysis, if the binding of substrates and enzymes is not

351 transitional (Sutton and Sposito 2005; Zang et al. 2000).

352   The HAA pools should contain soluble proteins; the soluble protein fraction is

353   estimated to be 4-9 % of HAA at the old site, 2-4 % at the intermediate site, and 1 % at the

354   young site, when average N content of proteins is assumed to be 17% of the mass (Sterner

355   and Elser 2002). Currently available colorimetric methods for soluble protein assays,

356   including the one used in this study, are known to have interference with humic substances.

357   Specifically, humic acids can increase absorbance of solution, which results in overestimation

358   of protein concentration (Roberts and Jones 2008). Thus, we cannot rule out the possibility

359   that the higher soluble proteins observed at the old site simply reflected high humic

360   substances in the soils at the old site.

361   Practically all amino sugars (as well as most soil proteins) originate from microbial

362   biomass, because of the relatively minor pool size of other sources such as arthropods

363   (Myrold 1998; Sterner and Elser 2002). Upon death of these organisms, these macro-

364   polymers are either 1) stabilized for the long term in soil by encapsulation with humic or

365   mineral components (Lipson and Näsholm 2001; Zang et al. 2000) or 2) depolymerized into

366   smaller fragments and available N by extracellular enzymes (Schimel and Bennett 2004).

367   Based on the absence of accumulation of HAS or HAA with increasing soil age, we suspect

368   that HAS and HAA constitute pools that turn over at an intermediate rate, because they

369   appear to be moderately stabilized but not completely protected from decomposition.

370   Furthermore, the significant differences in HAS:HAA ratios across soil ages suggest that N

371   dynamics that differ among sites are mediated by soil microbes.

372   **Fertilization and warming effects**

373   Fertilization had a large impact on readily-available N pools, but generally had little effect on

374   all other N pools. Total N added via fertilization was 11.4 mol/m^2 at the intermediate-age site

375   and 5.7 mol/m^2 at the young site. This N is equivalent to 54 (tussock) to 25 % (inter-tussock)

376   of native N at the intermediate-age site and 8 % (tussock) to 11 % (inter-tussock) of native N

377   at the young site. Nitrogen stored in plant biomass could account for only up to one-tenth of

378   the added N (~1.1 mol-N/m^2 at the intertmediate-age site and ~0.6 mol-N/m^2 at the young

379   site, Chapin et al. 1995; Hobbie et al. 2005). However, total soil N concentrations were

380   unaffected by this large N input (Fig. 3a), and even showed a trend toward decreasing total N

381   under the fertilization treatment at the intermediate-age site, suggesting that there was little

382   net retention of added N in the soil. Combined with the fact that fertilization significantly

383   lowered tussock-soil C:N ratios at both sites (young and intermediate-age), our results further

384   suggest that fertilization has caused a net loss of soil C in tussock soils. Our results are

385   consistent with recent findings. In a separate long-term fertilization study at the intermediate-

386   age site (yet in different subplot areas), in which N was added at the same rate as in this study

387   for 20 yrs, Mack et al. (2004) found that fertilization caused net losses of both soil C and N.

388   They concluded that increased nutrient availability had enhanced decomposition. Because

389   total N concentration did not differ across our treatments, our results imply that fertilization

390   allocated a greater fraction of total N to water-soluble N or gaseous N, resulting in the lack of

391   N accumulation in the fertilized soil. Leaching loss from the fertilized plots during the

392   growing season might be occurring mainly as available N (DIN) rather than as less available

393   N (DON). For example, fertilization significantly raised water-soluble DIN by as much as

394   100 -fold relative to the control, but DON was raised only doubled in tussock soil of the

395   intermediate-age site (Fig.3). Furthermore, during the peak growing season in July, DIN

396   concentrations in water leached to the bottom of the active layer or to micro-topographic

397   depressions were elevated by 100-200 times at both sites (Yano, unpublished data; the water

398   was collected using a syringe (Yano et al. 2010) from within or adjacent to the borders of the

399   fertilized plots). Based on these observations, we suspect that a significant portion of the N

400   loss might be occurring as DIN leaching during the growing season, bypassing plant and

401 microbial uptake. Perhaps, the plant-soil system in the fertilization plots has become N-

402 saturated after the many years of continuous fertilization.

403   Stronger fertilization effects on the soluble inorganic-N fraction found for the

404 intermediate-age site relative to the young site might be associated with native differences in

405 microbial processing of organic matter between these sites. Soil respiration, decomposition

406 rates, and dissolved organic matter production differed between the intermediate-age and

407 young sites (Hobbie et al. 2002). Differences in the effects of fertilization can also be

408 explained simply by the "cumulative effect" of greater total N added to the former site over

409 the longer time (intermediate-age site = 16 yrs, young site = 8 yrs). In a long-term

410 fertilization experiment on tussock tundra near our study sites, (Chapin et al. 1995; Shaver et

411 al. 2001) observed progressive changes in plant biomass, production, and plant-species

412 richness and composition over a 15-year study period. For example, aboveground biomass of

413 deciduous shrubs in N- and P-fertilized plots continued to increase through the study period,

414 whereas graminoids continued to decrease (Shaver et al. 2001). Differences in initial species

415 composition and an early-stage response to a chronic environmental change might also have

416 cascading effects on the status of soil nutrients that would set different response trajectories

417 in motion, over the long term. In the early stages of N and P fertilization, *Betula* became the

418 dominant species by the second year at the intermediate-age site, whereas the young site

419 (which had no *Betula* in the unmanipulated vegetative community) maintained its original

420 species composition relatively well for the first four years (Hobbie et al. 2005).

421   While the fertilization treatment specifically simulates the effects of expected increase

422 in N mineralization by adding inorganic N, the warming treatment simulates all the impacts

423 of raised air temperature, which is not limited to changes in N mineralization. Perhaps this

424 manifold effect is the reason that, in contrast with the fertilization that caused marked

425 changes mostly in soluble inorganic-N fraction, the effect of warming was smaller and

426 occurred only to properties associated with organic compounds (i.e., soluble AA and bulk soil

427 C:N ratio). The changes in C:N ratios with little changes in total or inorganic-N pools might

428 indicate that the N cycling remains tight under warming, while changes in temperature can

429 have a greater net impact on the C cycling in the tussock soils. The elevated C:N ratio in the

430 tussock by warming at the intermediate-age site was perhaps because of considerable

431 accumulation of deciduous litter at this site relative to the young site. The discrepancy

432 between effects of fertilization versus warming indicates that elevating nutrient availability

433 directly by fertilization versus indirectly by warming exerts different impacts on soil

434 biogeochemical processes. Perhaps this difference might be partly responsible for the large

435 differences found in composition of plant functional groups (Chapin et al. 1995; Gough and

436 Hobbie 2003) between the fertilization and warming treatments at these sites. A shift in

437 HAS:HAA ratios under fertilization but not warming suggests that long-term fertilization

438 might have altered microbially mediated N cycling.

439   Tussock soils generally responded more strongly than did inter-tussock soil to the

440 fertilization and warming treatments, at both intermediate-age and young sites. We suspect

441 that this differential response occurred partly because the treatments generally decreased the

442 productivity of the graminoid species, including *Eriophorum* but increased the productivity of

443 dwarf shrubs, which are more common in inter-tussock areas (Chapin et al. 1995; Gough and

444 Hobbie 2003; Shaver et al. 2001). Perhaps more-vigorous uptake in the inter-tussock area by

445 shrubs under the treatments lowered extractable available N, whereas reduced N uptake by

446 graminoids in tussock soils resulted in higher available N levels.

447   Both the fertilization and warming manipulation caused large declines of moss

448 biomass at the intermediate-age and young sites (Chapin et al. 1995; Gough and Hobbie

449 2000; Hobbie et al. 2005). Given mosses' properties such as slow decomposition (Hobbie

450 1996), preservation of surrounding organic matter (Børsheim et al. 2001; Painter 2003), and

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451 high nutrient and water retention capacity (Kotanen 2002) (Yano et al. 2010), replacement of

452 moss biomass with other plants because of a warmer climate would imply a significant

453 change in N cycling in arctic moist tussock-tundra ecosystems.

Conclusion

455   Readily available and moderately available N differed markedly among the three

456 tussock tundra ecosystems, while hydrolysable and total N concentrations were similar.

457 High soluble-AA dominance at the old site and high inorganic-N dominance at the young site

458 are clearly related to their contrasting soil-chemical properties, and might also be affected by

459 differences in vegetation composition and N uptake requirements.

460   Long-term fertilization and warming had different impacts on soil N pools in these

461 tussock tundra ecosystems, indicating that a warmer climate will likely have more-complex

462 impacts than a simple increase in N mineralization. The main effect of fertilization was a

463 several-fold increase in readily available N, with most of the increase occurring as inorganic-

464 N. Long-term fertilization also appeared to increase N losses from the soil via leaching. In

465 contrast, the effect of warming occurred only in tussock soils and only affected properties

466 associated with organic compounds, such as soil C:N ratio and soluble AA. Finally, our

467 results imply strong links between plant community composition and soil N dynamics in

468 arctic tussock-tundra ecosystems. Large declines of moss biomass under fertilization or

469 warming, a trend found consistently across various tussock-tundra sites, might have

470 significant impacts on N cycling in these arctic tundra ecosystems. The initial composition of

471 the plant community and soil chemical properties between soil types and among soil age are

472 important factors that merit consideration when projecting ecosystem-level response to

473 increased nutrient availability under a warmer climate. In northern Alaska, all of these

474 factors vary predictably with soil age, or time since deglaciation, indicating consistent and

475   predictable differences in the responses of different-aged landscapes to predicted climate

476   warming.

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Table 1. Aboveground live biomass and soil properties of three tussock-tundra ecosystems.

	Old		Inter-mediate		Young	
Aboveground live biomass						
Biomass distribution (% of total biomass) [a]						
Graminoids	3		9		21	
Deciduous	10		20		9	
Evergreen	11		34		15	
Forbs	0		0		1	
Lichens	0		9		13	
All mosses	76		28		40	
Total biomass (g/m^2) [a]	547		491		412	

Soil property	Tussock		Inter-tussock		Tussock		Inter-tussock		Tussock		Inter-tussock	
pH (Soil:H$_2$O = 1:2) [b]												
Organic soil (up to 15 cm)	4.0	(0.1)	4.9	(0.1)	4.0	(0.1)	4.6	(0.1)	6.9	(0.1)	7.0	(0.1)
Mineral soil (within top 15cm)	n.a.		n.a.		n.a.		4.8	(0.0)	7.0	(0.1)	6.9	(0.3)
Ca (mg/g) [c]			~8				3.1	(0.5)			25.3	(1.8)
CEC (cmol$^{(+)}$/kg) [c]			.				95.0				144.3	
Total C (%)	44.6 [b]	(0.2)	39.0 [b]	(1.7)	30.3	(4.2)	24.5	(7.8)	36.4	(2.7)	14.8	(3.9)
Total N (%)	0.7 [b]	(0.1)	1.3 [b]	(0.2)	0.9	(0.2)	1.0	(0.3)	1.5	(0.2)	0.9	(0.2)
Bulk soil C:N (molar)	76.7 [b]	(9.1)	32.5 [b]	(6.2)	42.1	(3.9)	27.8	(2.7)	29.7	(3.2)	19.5	(1.1)
Base saturation (%) [c]			.				19.8				71.3	
Moisture content (%)	63.8	(1.1)	59.4	(5.3)	52.1	(2.3)	56.8	(6.9)	48.3	(4.7)	44.4	(6.3)
Organic soil thickness (cm)	≥ 15.0	(0.0)	≥ 15.0	(0.0)	≥ 15.0	(0.0)	11.0	(2.0)	14.4	(0.5)	13.6	(0.6)

[a] Sources: Old = Hahn (1996) and Hastings (1989); Intermediate & Young = data by L. Gough & S. Hobbie available on Toolik LTER web site(http://ecosystems.mbl.edu/arc/datacatalog.html); MNT = Gough&Hobbie (2003).
[b] Soil collected in July 2012 and processed in the same manner as for the other soils.
[c] Determined for organic soil. Old = Walker & Walker (1989); Intermediate & Young = Hobbie & Gough (2002)
n.a.: not applicable.

Table 2. Statistical significance of soil-age and soil-type effects on biochemical parameters at the old, intermediate-age (Mid), and young sites. To test null hypotheses (H_0), contrast was used for target treatment means by fixing soil type to either tussock (T) or inter-tussock (IT). HAS= hydrolysable amino sugars; HAA = hydrolysable amino acids.

			Site age						Soil type
			Tussock			Inter-tussock			
	unit	H_0 :	Old = Mid	Old = Young	Mid = Young	Old = Mid	Old = Young	Mid = Young	T=IT
Bulk N	%		−	*	−	−	−	−	−
C:N (molar)			***	***	**	−	***	*	***
NO_3-N	µmol/g-dry soil		−	−	−	−	−	−	−
NH_4-N	µmol/g-dry soil		**	−	**	−	−	*	−
AA	µmol/g-dry soil		***	***	−	***	***	−	−
Soluble proteins	µg/g dry soil		***	***	**	**	***	−	***
Protease activity	µmol g^{-1} dry soil h^{-1}		−	−	−	−	−	−	*
Total Hydrolysable N	µmol/g-dry soil		−	−	−	−	−	−	−
HAS:HAA			−	**	**	*	**	***	−

*P<0.05; **P<0.01; ***P<0.0001; − lack of difference (P≥0.05).

Table 3. Statistical significance of treatment and soil type effects on biochemical parameters at the intermediate-age and young sites. To test null hypotheses (H_0), contrast of treatment means were used for Control (C), fertilization (F), and warming (W) treatments for each site by fixing soil type to either tussock (T) or inter-tussock (IT). HAS= hydrolysable amino sugars; HAA = hydrolysable amino acids.

			Intermediate-age Site						Soil type	Young Site						Soil type
			Treatment							Treatment						
			Tussock			Inter-tussock				Tussock			Inter-tussock			
	unit	H_0 :	C=F	C=W	F=W	C=F	C=W	F=W	T=IT	C=F	C=W	F=W	C=F	C=W	F=W	T=IT
Total N	%		–	–	–	–	–	–	–	–	–	–	–	–	–	–
C:N (molar)			*	*	***	–	–	–	***	**	**	–	–	–	–	–
NO$_3$-N	(µmol/g-dry soil)		***	–	***	***	–	***	–	*	–	*	**	–	*	–
NH$_4$-N	(µmol/g-dry soil)		***	–	***	**	–	**	*	–	–	–	–	–	–	–
DON	(µmol/g-dry soil)		*	–	**	–	–	–	–	–	–	–	–	–	–	***
AA	(µmol/g-dry soil)		**	**	–	–	–	**	*	–	–	–	–	–	–	–
Soluble proteins	(µg/g dry soil)		–	–	–	–	–	*	*	–	–	–	–	–	–	–
Protease activity	(µmol g^{-1} dry soil h^{-1})		–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total hydrolyzable N	(µmol/g dry soil)		–	–	–	–	–	–	*	*	–	–	–	–	–	–
HAS:HAA			*	–	*	*	–	–	–	*	–	*	–	–	–	–

*$P<0.05$; **$P<0.01$; ***$P<0.0001$; − lack of difference ($P\geq0.05$).

Figure Legends

Figure 1. Readily and moderately available N pools and protease activity across sites of varying soil age. Readily available N = water-extractable NH_4^+, NO_3^-, and AA (a); soluble proteins (b); and protease activity (c). Old = ~300k year-old acidic, moist-tussock tundra site in the Imnavait watershed, north-central Alaska, USA; Intermediate = 50-120k year-old acidic, moist-tundra site at Toolik LTER; Young = 11.5-25k year-old non-acidic, moist-tussock tundra site in Toolik LTER; T = tussock soils; IT = inter-tussock soils. All N pools were expressed per gram of dry soil. Error bars depict ±1 SE; n=3.

Figure 2. Hydrolysable-N pools and HAS:HAA ratio in soils of tussock tundra sites of different ages. Hydrolysable N pools (a) and HAS:HAA ratio (b). HAm = hydrolysable NH_4^+; HAS = hydrolysable amino sugars; HAA = hydrolysable amino acids. Error bars depict ±1 SE; n=3. See Figure 1 legend for the abbreviations.

Figure 3. Total N and C:N ratio of bulk soils and water-soluble N pools across treatments at the intermediate-age and young sites. Total N and C:N ratio (a); readily available N (b); soluble proteins (c); and DON (d). Cont = control; Fert = fertilization; Warm = warming. Error bars depict ±1 SE; n=2 for the warming treatment at the young site and n=3 for all others. See the Figure 1 legend for all other abbreviations.

Figure 4. Hydrolysable-N pools and HAS:HAA ratio in soils across the treatments at the intermediate-age and young sites. Hydrolysable N pools (a); and HAS:HAA ratio (b). Error bars depict ±1 SE; n=2 for the warming treatment at the young site and n=3 for all others. See Figure 1 and 3 legends for abbreviations.
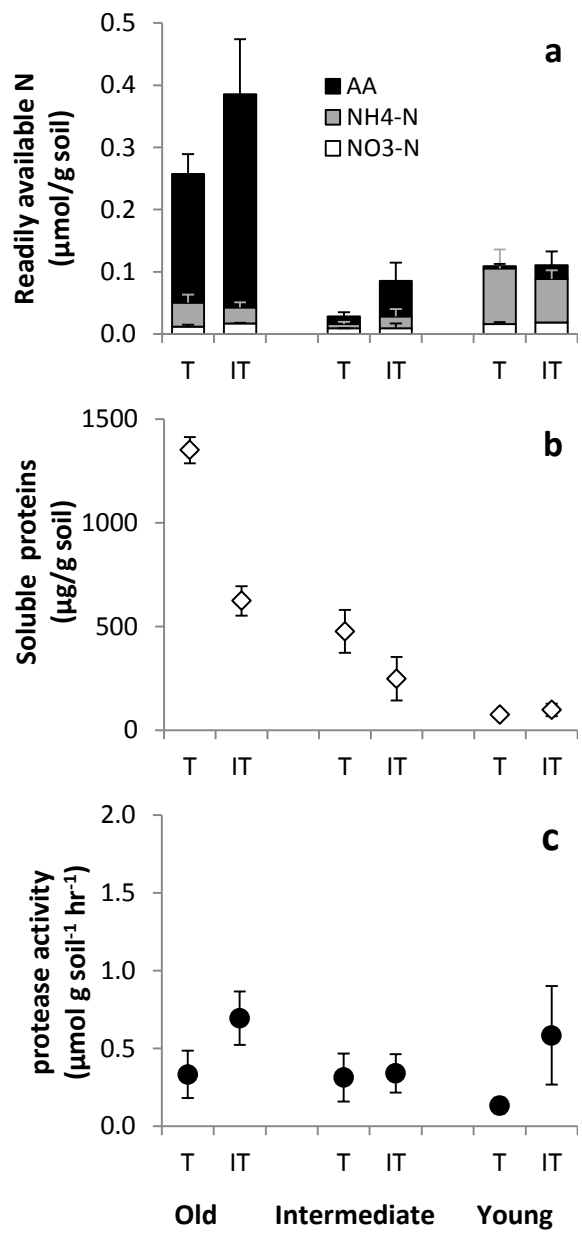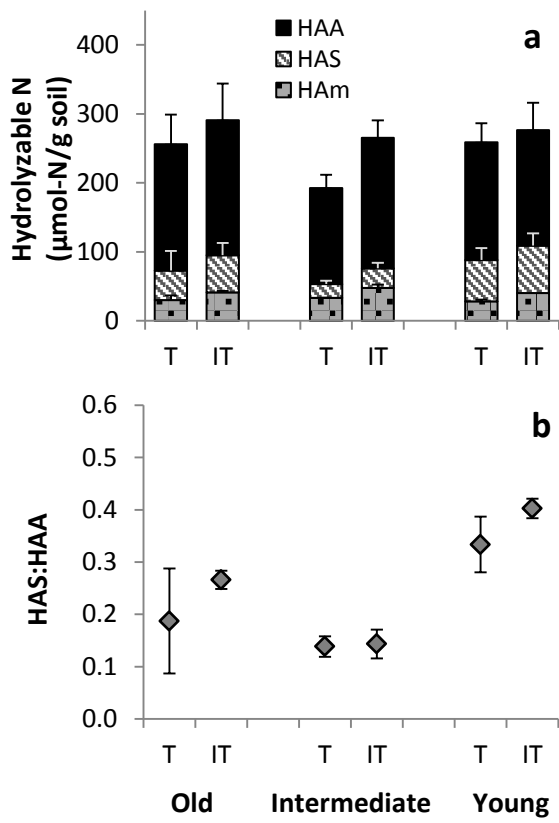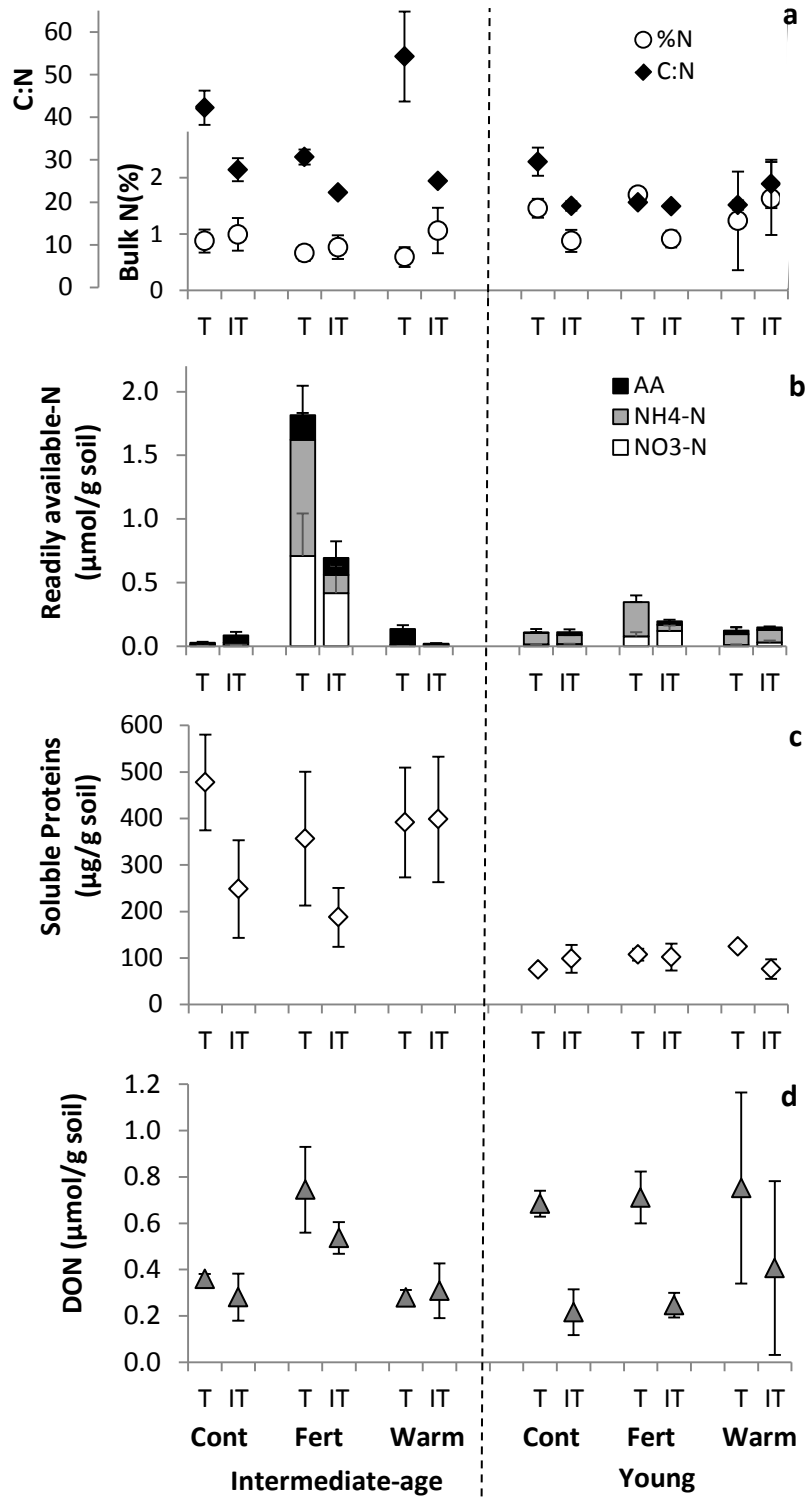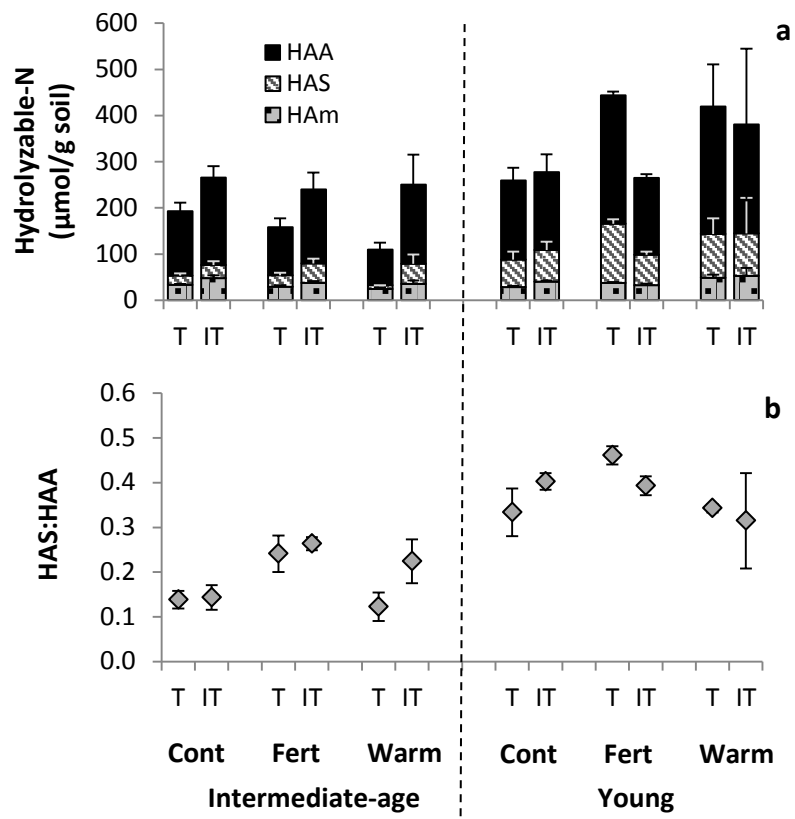
Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Appendix

Comparison of readily-available N and soluble protein pools in current and other studies. Data point(s) collected in mid-late July were chosen for comparison.

Site	soil type	NH_4^+	NO_3^-	AA	Soluble proteins	Source
			(µg/g)		(mg/g)	
Intermediate-age	Tus	0-1.5	0-2	0-1	1.5-2	Weintraub (2005) a,b[1]
		0.1	0.1	0.2	0.5	This study[2]
	IntTus	0-2	1.5-3	0-2	2.2-3.7	Weintraub (2005) a,b[1]
		3.0	0.5	1.3	-	Noridin (2004)[3]
		6.8	0.2	-	-	Hobbie & Gough (2003)[4]
		0.3	0.1	0.8	0.25	This study[2]
Young	Tus	1.3	0.2	0.1	0.08	This study[2]
	IntTus	7.0	0.8	3.5	-	Noridin (2004)[3]
		6.2	0.7	-	-	Hobbie & Gough (2003)[4]
		1.0	0.3	0.3	0.10	This study[2]

Extraction methods and data points compared:

[1] 0.5M K_2SO_4 for NH_4^+ and NO_3^-, water for AA, 0.1M $NaHCO_3$ for proteins; a range of late-July data points were used

[2] 0.1M $NaHCO_3$ for proteins, water for the rest

[3] 2M KCl; Average of July and August of O-horizon soils were used

[4] Water; July data were used

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