

CARBON TURNOVER IN ALASKAN TUNDRA SOILS: EFFECTS OF ORGANIC
MATTER QUALITY, TEMPERATURE, MOISTURE, AND FERTILIZER

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Running headline: CARBON TURNOVER IN TUNDRA SOILS

SUMMARY

- 1.) Northern ecosystems may lose large amounts of soil C as the global climate warms over the next few decades. This study describes how soil C loss is related to temperature, moisture and chemical composition of organic matter in Alaskan tundra soils, including soils that were fertilized annually for eight years prior to the study.
- 2.) Fertilized and unfertilized soils from four vegetation types (tussock, intertussock, sedge, and heath) were incubated at 7° or 15 °C, and under saturated or well-drained conditions, through four 100-day “seasons” separated by 25-45 day frozen periods.
- 3.) Losses of CO₂ were monitored and total C loss was determined by difference between initial and final C stocks. Initial and final organic matter composition was determined by separation into non-polar extractable (NPE; mainly fats, oils, and waxes), water-soluble (WS; mainly soluble carbohydrates and phenolics), acid-soluble (AS; mainly cellulose and related compounds), and acid-insoluble (AIS; “lignin”) fractions. An isotopic label (99% ¹³C-enriched glucose) was added to track transformations among the C fractions.
- 4.) Total C loss during the experiment was 3-32% of initial C mass depending on soil type and treatment, with most of the loss as CO₂. Wet sedge tundra soils, with the largest AS and AIS fractions, lost the least CO₂ and total C. The added ¹³C ended up in all C fractions, indicating production, as well as loss of all fractions.
- 5.) The greatest CO₂ and total C losses occurred under warm, well-drained conditions, in all soils. The effects of fertilizer treatment were occasionally significant but never large relative to the other treatments.

- 6.) Despite the long incubation under standard conditions, there was no evidence for convergence in C chemistry among soils as indicated by changes in relative abundances of the four C fractions.
- 7.) Large and constant rates of C loss even after 4 “seasons” of incubation suggest that a large portion of the C pool is potentially mineralizable in all soil types.
- 8.) Warming of the Arctic climate and associated thawing of permafrost and the increase in soil drainage have the potential to cause a large release of C. This C, currently stored in soil organic matter, will be released to the atmosphere creating a positive feedback on future climate changes.

KEYWORDS: Soil organic matter, soil carbon, peat, organic matter quality, proximate carbon fractions, decomposition, soil respiration, tundra, Alaska, climate change, greenhouse warming

INTRODUCTION

Soils of tundra and boreal ecosystems contain large organic matter stocks, typically as a layer of peat that blankets the underlying mineral soil. Despite the low productivity of northern vegetation, organic matter accumulates as peat because decomposition of plant litter is limited by low soil temperatures and often wet, anaerobic conditions (Heal et al. 1981, Jonasson et al. 2001). The total C storage in this northern peat is globally significant, accounting for about one third of the global soil C stock if one includes both tundras and boreal forests (Oechel and Billings 1992, Callaghan et al. 2004a). Soils of northern ecosystems also contain large amounts of organic N that is currently unavailable to plants, but is potentially available and could support higher productivity if mineralized (Shaver et al. 1991, Nadelhoffer et al. 1992, Weintraub and Schimel 2005 a).

Controls on soil C stocks and turnover, therefore, are key issues for understanding C exchanges between northern ecosystems and the atmosphere. In this paper, we determine how C losses from peaty soil organic matter are related to its chemical composition, and how that composition changes as the organic matter decomposes. To address these issues we compared four soil organic matter types from three tundra ecosystems near Toolik Lake, Alaska. The comparison included both unfertilized soils and soils that were fertilized annually for eight years before sampling. Under laboratory conditions, we determined how temperature and moisture conditions affect C losses from these organic matter types. The experiment also allowed us to determine how the chemical composition of different types of organic matter changed over four simulated “seasons” of decomposition.

The chemical composition or “quality” of soil organic matter is a useful predictor of C turnover (Ågren and Bosatta 1996) although a wide range of definitions and fractionation

schemes have been used (Sollins et al. 1999, Harmon and Lajtha 1999). In general, high-quality organic matter is defined as that which is more readily processed by microbes and has a higher rate of decomposition. Fresh plant litter and newly-formed organic matter are expected to be of higher quality than older, more fully decomposed organic matter in which the more labile components have been metabolized (Aerts 1997, Berg 2000). Species composition of the vegetation may also have a strong influence on litter and organic matter “quality” (Berendse 1994, Cornelissen 1996, Hobbie 1996, Hobbie and Gough 2004). In this research we characterized organic matter quality with a widely used sequential extraction procedure (Ryan et al. 1990, Harmon and Lajtha 1999) that breaks soil organic matter into 4 fractions: (1) a “non-polar extractable” (NPE) fraction extracted in methylene chloride, (2) a “water-soluble” (WS) fraction extracted in boiling water, (3) an “acid-soluble” (AS) fraction extracted in H₂SO₄, and (4) an “acid-insoluble” (AIS) residue.

EXPERIMENTAL DESIGN AND METHODS

Field sites, experiments, and soils

The soils used in this research came from the Long Term Ecological Research (LTER) site at Toolik Lake, Alaska (68°37'N, 149°36'W, elevation 720 m), a site that has been studied extensively since the mid-1970s (e.g., Shaver and Chapin 1991, Walker et al. 1994, Shaver 1996, Hobbie et al. 2003). Annual average temperature is approximately -7°C ; thus the area is underlain by continuous permafrost. Although frosts can occur at any time, air temperatures are generally above freezing in June, July and August; the average temperature in July is 10°C . Soil temperatures during the summer decline steeply with depth, from $10\text{-}20^{\circ}\text{C}$ or more at the surface on a sunny day, to 0°C at the bottom of the seasonally-thawed, “active layer.” The thickness of this thawed layer, and the temperatures within it, vary greatly among ecosystem types and among years but average 30-60 cm in late July. Although the soils are frozen during the winter months, insulation by snow cover, prevents soil temperatures from reaching the extremes experienced aboveground ($<-30^{\circ}\text{C}$); winter soil temperatures at 10 cm depth average -5 to -8°C .

The ecosystem types selected for the present study included:

- (1) dry heath tundra (hereafter, “heath”), dominated by evergreen dwarf shrubs and lichens with a thin (1-5 cm) organic mat overlying a deeply-thawed ($>1\text{m}$) rocky glacial outwash soil (Shaver and Chapin 1991, Gough et al. 2002);
- (2) wet sedge tundra (“sedge”), dominated by rhizomatous graminoid species with a thick organic mat (25-30 cm), the presence of surface water for most of the summer, and an annual soil thaw depth of 40-60 cm (Shaver et al. 1998, Johnson et al. 2000, Boelman et al. 2003);
- (3) moist acidic tundra, composed of a mix of deciduous, evergreen and graminoid species (Shaver and Chapin 1991, Shaver et al. 2001, Gough et al. 2000, Hobbie et al. 2005). The

organic mat thickness ranges from <5 to >40 cm but averages about 15-20 cm, with a summer thaw depth of 40-60 cm. Because spatial heterogeneity in this tundra is largely created by the sedge, Eriophorum vaginatum (Chapin et al. 1979), we sampled separately the soils within and beneath tussocks (“tussock”) and the soils between the tussocks (“intertussock”).

Thus, there were four organic matter types compared in this study; i.e., heath, sedge, tussock and intertussock. The sedge, tussock and intertussock soils were similar to those studied by Weintraub and Schimel (2003, 2005a, 2005b) and were collected from the same areas within 200-300 m of the south shore of Toolik Lake.

All four soil types were collected from control plots and from plots that had been fertilized annually for 8 years with $10 \text{ g N m}^{-2} \text{ y}^{-1}$ (as NH_4NO_3) and $5 \text{ g P m}^{-2} \text{ y}^{-1}$ (as P_2O_5). In all sites, the N and P were added each June as granular agricultural fertilizers, as in over 20 previous experiments in northern Alaska (e.g., Shaver and Chapin 1995). By the time of sampling in 1997, fertilization had caused major changes in species composition and productivity at all sites (Shaver et al. 1998, 2001, Gough et al. 2002). In addition to the direct input of fertilizer N and P, leaf litter inputs had increased 1.5-5 fold, moss cover was much reduced or eliminated, and the root mass had increased (Nadelhoffer et al. 2002, Mack et al. 2004).

Soil collection and initial processing

Soils were collected in late August 1997 using a 5.08 cm diameter corer in the sedge, tussock and intertussock soils, and a 10.16 cm diameter corer in the heath soil. The corer was pushed through the peaty upper organic mat, and the depth of the hole was recorded to account for compression of the peat during the coring. All cores were refrigerated during shipment to Woods Hole, Massachusetts, where they were stored at -4°C .

The soil cores were thawed and prepared for incubation in July 1998. For each core, the entire upper organic mat (upper 20 cm in sedge soils) was homogenized after the live plant material (roots and rhizomes) was removed. A subsample was then analyzed for organic content and C, N and P content. Total organic content was determined by ashing at 500 °C, and C and N content were determined with a Perkin-Elmer Elemental Analyzer. Total P was determined by the methods of Aspila et al. (1976) as modified by Ruttenberg (1992).

A second subsample of the homogenized organic matter was analyzed using the proximate carbon fractionation procedure of Ryan et al. (1990). This procedure yields four organic matter fractions by sequential extraction: non-polar extractives (NPE), hot water-soluble (WS), acid-soluble (AS) and acid-insoluble (AIS). Subsamples for fractionation consisted of approximately 2 g of finely ground, oven dried (50°C), homogenized soil. Each subsample was taken through three sequential extractions, with residual soil dried at 50°C for 48 hrs, weighed, and sampled for C, N and total P analysis. Methylene chloride (70 ml) was used to extract NPE compounds, including fats, oils and waxes. The remaining soil was extracted with ~75 ml hot water to remove the WS fraction containing bioactive carbohydrates and soluble phenolics. Finally, 72% sulfuric acid was used to remove the AS fraction (mainly cellulose and related compounds). The remainder was considered to be the AIS fraction, containing mainly lignin and related compounds. Concentrations of all fractions were corrected for ash content.

Incubation experiment

Homogenized organic matter from each soil type was incubated in Conviron PGV36 growth chambers for ~465 days, including 4 simulated “seasons” of 100 days each. Each “season” included 70 days at constant 7° or 15°C, with a 15-day warm-up and cool-down period. Between “seasons” the soils were frozen for 4-6 weeks at -4 °C. A temperature of 7°C is within

the normal summertime range for the upper 10 cm in all of these soils, although there is considerable daily, seasonal and depth-related variation. Heath soils tend to be warmer and sedge soils tend to be colder than 7°C. The 15°C regime was chosen because the 8° C increase was large enough that we would expect a significant and measurable temperature response (Nadelhoffer et al. 1991) but still within the upper bounds of expected temperature increases in a future, warmer climate (ACIA 2004). The “winter” temperature of -4°C was the coldest temperature that could be maintained by these growth chambers, but was similar to temperatures in the upper 10 cm in the field, for much of the wintertime, except perhaps in the heath site where there is little or no insulating snow cover (G. Shaver and J. Laundre unpublished data). There were also two moisture treatments; in the “wet” treatment, the soils were not allowed to drain, with the water level kept at the soil surface by the addition of deionized water. In the “moist” treatment, soils were drained to field capacity and then kept at the same weight throughout the experiment by the addition of deionized water. The two temperature and two moisture treatments were applied factorially to all 8 soil types (7W=7°C, wet; 15W=15°C, wet; 7M=7°C, moist; 15M=15°C, moist).

Soils were incubated in 5.08 cm diameter core tubes (3.8 cm for heath soils) made of polyvinyl chloride (PVC). The tubes were capped at the bottom, with a screen-covered sample port for solution sampling and drainage control. Four tubes containing the control soils and 3 tubes containing fertilized soils were assigned to each treatment (7W, 15W, 7M and 15M). Between measurements the tubes were covered with loose foil caps to reduce moisture loss. Initial fresh weight of the incubated organic matter varied from 24-298g, depending primarily on the thickness of the upper organic mat in each core. Initial dry weights were 5-80g, estimated by dividing the wet weight by the wet:dry mass ratio as determined from subsamples of the initial

homogenized soils. At the end of the experiment, the remaining organic matter in each tube was dried, weighed, and analyzed by the same methods as the initial homogenized soils.

Isotopic labeling

Trace amounts of ^{13}C (as 99% ^{13}C -enriched glucose) were added to two tubes of each of the unfertilized soils at the start of the incubation. The glucose was dissolved in water and applied in volumes that varied depending on the initial estimated soil dry mass. For example, tubes from the heath site, containing soil that weighed approximately 20g on average, received an average of 0.0238g of ^{13}C ; and the sedge site soils, weighing an average of 45g, received an average of 0.0595g of ^{13}C . To determine the fate of the ^{13}C , at the end of the incubation, each of the four C fractions from each labeled core was analysed using a Finnegan Delta S isotope ratio mass spectrometer (Fry et al. 1992).

C losses

Losses of C as CO_2 were measured 10 times per “season,” at the beginning and middle of the warm-up period, at the middle and end of the cool-down period, and 6 times (every 2 weeks) during the “season”. The “winter” CO_2 flux was measured at -4°C after the first growing season, and this flux was assumed to be the same in all frozen periods. The CO_2 flux measurements were made using a LiCor LI6200 infrared analyzer attached to the incubation tubes to form a closed loop from the tube through the analyzer (Johnson et al. 1996). The flux is calculated as a result of knowing the rate of increase in CO_2 concentration in a closed system, the volume of airspace above the soil, the mass of the soil, temperature and barometric pressure. Time-integrated losses of $\text{CO}_2\text{-C}$ were calculated for each core by linear interpolation between successive measurements.

Total C losses were calculated from the changes in total core mass and C content. The initial dry mass of each core was estimated using wet:dry mass ratios determined from subsamples of the initial homogenized soils. Initial C mass and masses of each C fraction were calculated from total C and organic matter percentages of the dry mass. Overall losses were estimated by subtracting the measured final mass from the initial estimates.

Statistical analysis

Statistical analyses were completed using SYSTAT 8.0 and 10.0 software (© SPSS Incorporated, 1998). The principal analyses were factorial analyses of variance (ANOVA) by soil type (H, S, T, or I), with fertilizer treatment, moisture and temperature as factors. Where appropriate the data were log-transformed to achieve homogeneity of variance. Results of the C fraction analysis for both initial and final chemical composition, were analyzed using ANOVA for individual C fractions, and discriminant analysis and MANOVA (DISCRIMINANT and ANOVA procedures in SYSTAT) to compare overall differences among soils from the beginning to the end of the incubation. In the multivariate analyses, the amounts in each C fraction were calculated per gram of bulk soil, rather than per gram of organic matter.

RESULTS:

Homogenized soils before incubation

The soils varied in their C, N, and P concentrations (Table 1). C concentrations ranged from 28-38%, with %C in fertilized intertussock soils slightly lower than controls and %C in fertilized tussock soils slightly higher than controls. Total C and N concentrations were much higher in the sedge soils (38% and 2.5%) than in the other three soils. There was little or no effect of fertilizer on N concentration although N concentration was slightly *lower* in fertilized intertussock soils than in controls. Total P varied from 0.85-1.20 mg g⁻¹, with higher concentrations in fertilized soils.

The homogenized soils also differed in concentrations of the four C fractions (Fig. 1, Table 2). The largest of the fractions was consistently either the acid-insoluble (AIS) fraction or the acid-soluble (AS) fraction, and the smallest was always the nonpolar extractable (NPE) fraction. The water-soluble (WS) and NPE fractions were the most variable among sites. The sedge soils stood out for their high total organic matter concentrations (sum of the four fractions) and for their low concentrations of WS. Overall, although fertilizer effects and soil type x fertilizer interactions were significant for all but the WS fraction, the magnitude of the fertilizer effect was small relative to the differences among soil types (i.e. sums of squares for the soil type effect explained about three times as much of the variance). The differences among the 8 soils are shown clearly in a discriminant analysis (Fig. 2). The first discriminant function (horizontal axis) explains 63% of the variance and clearly distinguishes the sedge soils from all others. Distribution along this axis is correlated with high AIS and AS concentrations (box in Fig. 2). The second discriminant function (vertical axis) explains an additional 17% of the variance and is correlated with high NPE concentration. This second function clearly separates control from

fertilized tussock and intertussock soils, reflecting the very small NPE fraction in the fertilized soils (Fig. 1).

CO₂-C Loss

Integrated CO₂-C losses ranged from <2% to >30% of initial C content (Fig. 3), and treatment effects were highly significant for all soil types (Table 3; differences among soil types and soil type x treatment interactions were also highly significant in a 4-way ANOVA, results not shown). CO₂ loss during the “winter” periods was always less than 5% of total CO₂ loss, due both to the low respiration rates at -4°C and to the relatively short frozen period. Although respiration during winter can be significant on an annual basis (e.g., Zimov et al. 1996), the rate of respiration at -4°C is 1-2 orders of magnitude lower than at 7°C or 15°C (Mikan et al. 2002). The sedge soils had the lowest CO₂-C losses in all treatments, but all four soils had higher CO₂-C losses at 15°C than at 7°C and higher CO₂-C losses in the M than in the W treatments (temperature effects were not significant in Heath soils). There was also a temperature x moisture interaction in all soils (not significant in Heath), because the effect of warming was smaller in W than in M treatments. Fertilizer had little or no effect on CO₂-C loss except in tussock soils, where CO₂-C losses from fertilized soils were greater than controls in M treatments, but not in W treatments, leading to a significant fertilizer x moisture interaction.

C mass loss

Total C losses were calculated as the difference between the initial and final C masses of each core, and ranged from less than 4% to nearly 30% of initial C mass (Fig. 4). The tussock and intertussock soils consistently lost the most total C, while the heath soils were intermediate and the sedge soils lost the least C. Higher temperature and lower moisture consistently caused

greater C losses, with a significant temperature x moisture interaction in the heath and tussock soils (Table 3). Fertilizer effects were also significant in three of the four sites, but the fertilizer treatment *reduced* C losses relative to controls in the tussock and intertussock soils and *increased* C losses in the sedge soils (soil type x fertilizer interaction significant at $P < 0.001$ in the 4-way ANOVA, results not shown).

Total C losses were consistently greater than or approximately equal to CO_2 -C losses, with the difference potentially accounted for by methane (CH_4) losses and dissolved C losses in water drained from the cores (Johnson et al. 1996). Although we did measure both CH_4 -C and dissolved organic C (DOC) losses, neither is reported because the DOC losses were consistently small (less than 2% of initial C mass), and the CH_4 -C measurements were compromised by frequent high values, probably due to bubble emission. Estimated CH_4 -C losses were greatest in the sedge soils, however, particularly in the 7W and 15W treatments where there is a large difference between CO_2 -C losses and total C losses (Fig. 3 vs. Fig. 4; CH_4 data not shown)

Final soil C fractions

By the end of the experiment, the concentrations and relative abundances of the four C fractions had changed considerably. However, because the individual fractions changed similarly in all soils and treatments, it is difficult to discern any consistent or large differences due to fertilizer, temperature, or moisture treatment by determining the concentrations of the fractions. The overall pattern of change in the soil C fraction is clearer in a comparison of their relative changes in mass (Fig. 5). In this comparison, the NPE fraction increased in mass by at least 75% in every soil type and treatment. The increases in NPE were unaffected by temperature or moisture, but in the intertussock and tussock soils there was a strong fertilizer effect (Table 4), leading to 3-5 fold greater NPE accumulation in the fertilized soils. In contrast,

the WS fraction decreased in mass over the course of the incubation, in every soil type and treatment. The decreases in WS mass were also large, more than 50% in most cases, and were unaffected by temperature, moisture or fertilizer treatment. The only exception was in heath soils, where the fertilizer caused greater WS losses (Table 4).

The patterns of change in AS and AIS mass were more complex but in most soils and treatments these fractions lost mass (Fig. 5, Table 4). In the heath, sedge and tussock soils, the fertilizer caused larger losses of AIS than in the control samples. The fertilizer caused either less AS loss or more AS accumulation in the heath and sedge soils while in the tussock soils there was greater AS loss in fertilized treatments.

Overall, there was no evidence for convergence in composition of the organic matter among soil types, even after the equivalent of four full seasons of decomposition. Instead, the initial differences among soils were maintained even as all soils in all treatments tended to accumulate NPE, to lose much of their WS, and to remain relatively constant or (usually) to show small decreases in AS and AIS (Fig. 5). The lack of convergence is shown clearly in a discriminant function analysis comparing the composition of the initial soils with that of the post-incubation soils from the four temperature x moisture treatments (Fig. 6). In this analysis, the first discriminant function accounted for 71% of the total variance in the data set and separated the groups in a sequence that was nearly identical to the analysis of the initial soils only; that is, the first discriminant function was positively correlated with the AIS fraction, with the SC and SF soils at the far right, the TF and HC soils in the center, and the others to the left (compare Figs. 2 and 6). The second discriminant function accounted for an additional 21% of the variance and was positively correlated with the NPE fraction and negatively correlated with the WS fraction, i.e. the two fractions that changed the most in all soils and treatments (Fig. 5).

Thus, the first discriminant function clearly distinguished among the 4 soil types with or without fertilizer, mostly on the basis of AIS content, while the second function distinguished all post-incubation soils from all pre-incubation soils, mostly on the basis of NPE and WS content (Fig. 6). Neither of these two functions (which together accounted for 92% of the variance in the data) was effective at distinguishing the 4 temperature and moisture treatments within any of the 8 soils. As a result, the group centroids representing the post-incubation temperature and moisture treatments are tightly clustered by soil type and in all but one of 32 cases (one of the TF treatments) they are above and to the right of the corresponding group centroid, representing the initial soil composition.

Fate of added ^{13}C

Total recovery of ^{13}C at the end of the incubation ranged from 8-17% of the amount added (Fig. 7; only control soils were labeled). In all the soil samples, less ^{13}C was recovered in the 15°C treatments than at 7°C, probably due to higher respiration at the higher temperature. The temperature effect was highly significant ($P < 0.001$) in a 3-way ANOVA with site, temperature and moisture as main effects (data not shown) and was significant to at least $P < 0.07$ in all four ANOVAs by soil type (Table 5). Moisture had no large effect on ^{13}C recovery although in the intertussock soils ^{13}C recovery was slightly higher in the W treatments ($P < 0.05$).

The ^{13}C label was recovered from all C fractions in all soil types and treatments, with only two exceptions (Fig. 7). The exceptions were the NPE fraction in heath soils in the 7M treatment, and in tussock soils in the 15M treatment, where in both cases ^{13}C levels were not significantly lower than background leading to a negative estimate of ^{13}C accumulation in NPE. In general, ^{13}C recovery in the NPE and AIS fractions was greater at 7°C than 15°C and significantly so in the W than the M treatments, in the heath and intertussock soils (Table 5). In

contrast, ^{13}C recovery in the WS and AIS fractions was greater at 15°C than at 7°C and in the M than in the W treatments, particularly in the heath and intertussock soils.

DISCUSSION

Site differences predominate

The heath, sedge, tussock, and intertussock soils differed greatly in their C loss over four “seasons” of incubation, with among-site differences generally greater than within-site responses to temperature, moisture and fertilizer treatment. Although we would expect the microbial communities of the different soil types to be somewhat different initially due to their different soil environments as well as their different organic matter composition in the field, the long incubation time under identical temperature, moisture, and fertilizer conditions should, if anything, have promoted convergence rather than divergence in community composition by the end of the experiment. Thus, the most likely explanation for the large among-site differences in C loss is the differences in chemical “quality” or decomposability of the organic matter in the four soils. Also contributing to among-site differences in C loss may be differences in soil texture and mineral content, which could affect soil water movement, ion diffusion, soil redox status and anaerobiosis.

Differences in chemical quality of soil organic matter have two main causes. First, differences in species composition of vegetation may lead to differences in the chemistry of the leaf, stem and root litter that eventually becomes soil organic matter (Hobbie 1992, Aerts 1997, Hobbie et al. 2000). In an earlier survey of litter decomposition at Toolik Lake, Hobbie (1996) found that “differences in rates of litter decomposition were more related to carbon quality than to nitrogen concentration”. Based on Hobbie’s (1996) species-by species analyses of leaf, stem and root litter, we would expect the graminoid-dominated sites (tussock and sedge) to have higher initial AS content and lower AIS in their litter, in comparison to the intertussock and heath sites, because the tussock and sedge vegetation contains relatively little shrub biomass.

(Litter from both evergreen and deciduous shrubs has lower AS and higher AIS than graminoids.) However, in all four soils, the initial organic matter analyzed in our experiment was significantly different from any litter analyzed by Hobbie (1996); in our study the organic matter content was always higher in AIS and lower in NPE than in the litter fraction. With the exception of the sedge site, our initial organic matter was also higher in WS than in any of Hobbie's (1996) litter types. Only AS in our initial organic matter was within the range of AS as reported by Hobbie.

The range of variation in NPE, WS, AS, and AIS content in the initial organic matter (both control and experimental samples) was narrower than the range among fresh litters reported by Hobbie (1996). This reduced variability among organic matter types suggests considerable convergence in chemistry as fresh litter becomes soil organic matter. This convergence is consistent with the "litter decay continuum" concept (Berg 2000), in which AIS increases in relative abundance over time as a result of the preferential degradation of more labile substrates.

The second major cause of among-site variation in quality of soil organic matter is differences in temperature, moisture, pH and aeration. We already know that these variables differ greatly among sites at Toolik Lake (Chapin et al. 1979, Giblin et al. 1991, Shaver et al. 1998, 2001). Such differences should lead to differences in the rates of transformation of individual C fractions, as well as differences in overall C loss as fresh litter becomes soil organic matter (Hobbie 1996, Aerts, 1997, Weintraub and Schimel 2003, Hobbie and Gough 2004). Thus, even if fresh litter inputs were the same in all four sites, we would expect organic matter composition to differ as a result of decomposition in different environments.

As Hobbie (1996) found for fresh litter, we found that the soils with the largest AS and AIS fractions lost the least C. Most of this difference, however, was explained by the large differences between the sedge soils and the other three soils, with no correlation between C loss and concentration of any C fraction when the sedge soils were excluded. Weintraub and Schimel (2003), also working at Toolik Lake, found that sedge soils had lower respiration rates than other soils; in their study they found a positive correlation between CO₂ losses and WS, and a negative correlation between CO₂ loss and AIS:N ratio.

Although the high AIS in sedge soils may explain the low C losses during incubation, the initial litter inputs to the sedge soils were almost certainly low in AIS, as the wet sedge vegetation is strongly dominated by graminoids (Hobbie 1996) and contains relatively few, poorly-decomposable, but low-AIS mosses (Shaver et al. 1998). The question remains as to how such high AIS developed in the sedge soils but not in the others, which have higher AIS inputs in their more woody litter. The likely explanation is that environmental conditions of the sedge soils lead to an accumulation of AIS, either by inhibition of decomposition of AIS in the initial litter or by accumulation of AIS produced by microbes during decomposition, or both.

Lack of convergence in C chemistry during incubations

In constant environments such as those in our incubations, we expected further convergence in organic matter chemistry as microbes selectively processed the C fractions, forming organic matter with relatively less labile C (such as WS) and relatively more recalcitrant C (such as AIS). Eventually, a relatively stable composition should be reached, reflecting a slow overall C turnover and tighter linkage of the processes associated with the breakdown of the remaining organic matter. This more stable composition should also reflect the production and turnover of new C substrates that are products of microbial growth, death and metabolism (Berg

2000). Instead, we found that among-site differences in C chemistry were maintained despite the long incubation time, the common treatment conditions, the large changes in individual C fractions, and overall C losses of 10-30% in most soils. Even if we exclude the sedge soils from the analysis and focus on the most responsive (15M) treatment, there is no evidence for convergence in chemical composition.

The lack of convergence in C chemistry may have several causes. Firstly, the lack of convergence is consistent with later stages of the “decay continuum” as discussed by Berg and others (Berg 2000, Weintraub and Schimel 2003) in which microbes attack the available substrates roughly in proportion to their availability, rather than in sequence from highly labile sugars to highly recalcitrant lignin. Decomposition in proportion to availability would tend to maintain the relative differences in composition even with large overall C losses.

Secondly, the fact that the added ^{13}C ended up in all four fractions, and that the NPE fraction *increased* in all soils and treatments, indicates that all four soils had reached the point where overall decomposition was regulated mainly by breakdown of recalcitrant lignin and lignified carbohydrates (Berg 2000) and the pool sizes of the fractions were determined as a balance of new production and loss. In this state, microbial consumption of organic matter, followed by exudation and turnover of microbial bodies, represents the major C turnover processes and are new sources of all four C fractions, including chitins and other cell wall components that appear in the AS and AIS fractions, as well as microbial (mostly fungal) lipids and waxes that appear in the NPE fraction.

Finally, although we did see rapid declines in respiration over the first simulated season or two, indicating rapid depletion of a small labile pool, the steady respiration rates thereafter suggest that respiration was not limited by overall C availability but rather by the chemical

characteristics of a large, relatively recalcitrant C pool (for brevity, temporal trends in CO₂ losses are not shown in detail). Although temperature and moisture treatments did affect this long-term, stable respiration rate in all soil types, there was no evidence for depletion of this pool, or for switching to a different pool, even in treatments with the highest overall C losses (the 15M treatment). Both Hobbie et al. (2002) and Weintraub and Schimel (2003) came to similar conclusions in their incubations of soils from Toolik Lake.

Responses to temperature and moisture

Responses to the temperature and moisture treatments were generally as expected from previous research on similar soils, including soils from near Toolik Lake (e.g., Nadelhoffer et al. 1991, Hobbie et al. 2002, Mikan et al. 2002, Weintraub and Schimel 2003). CO₂ fluxes accounted for most of the total C losses, and the general pattern was of an increase in CO₂-C loss of 50-100% as temperature increased from 7⁰C to 15⁰C, and a decrease in CO₂-C loss of 50-75% going from freely-drained to saturated moisture conditions. Temperature sensitivity of CO₂ loss was greater under drained conditions than under saturated conditions, as observed by many others (e.g., Flanagan and Veum 1974). Effects of temperature and moisture on individual C fractions were generally small although occasionally significant. Because the temperature difference between treatments (8°C) was near the high end of expected climate warming scenarios (ACIA 2004), and because the moisture treatments represented extremes of drainage (saturated and freely-drained), we would expect responses to actual climate change in the coming decades to be somewhere in between the extremes observed in this experiment.

Effects of fertilizer

Although fertilizer effects on initial C chemistry, on C losses during incubation, or on final C chemistry were often significant statistically, the magnitude of those effects was small relative to the much larger effects of soil type, temperature and moisture. The only large fertilizer effects were in the tussock and intertussock soils, where the fertilizer-treated soils lost 20-50% less C mass than the control soils. In addition, the fertilized tussock and intertussock soils had the lowest initial NPE concentrations among all soils (Fig. 1) and the greatest initial difference between fertilized and control soils in the discriminant analysis (Fig. 2). Over the course of the incubation, the fertilized tussock and intertussock soils exhibited a 3- to 6-fold increase in the NPE fraction than the controls (Fig. 9). Because these fertilized soils were initially lower in NPE, the larger increase in NPE in fertilized soils, if anything, would have contributed to convergence in C chemistry in the tussock and intertussock soils in the final discriminant analysis (Fig. 10). The reason for the effects of pre-incubation fertilizer addition on NPE accumulation in these two soils is not clear although the difference must reflect some difference in microbial metabolism or community composition.

Schimel and Weintraub (2003; Weintraub and Schimel 2005b), based in part on their work at Toolik Lake (Weintraub and Schimel 2003), suggest that reduced C losses from fertilized soils relative to the controls may reflect N-limitation of microbial growth, as C is redirected from waste respiration to microbial growth when additional N is available. In other studies, N addition has had a wide range of effects on C turnover (e.g., Neff et al. 2002, Giardina et al. 2004, Pregitzer et al. 2004). Our study differed, however, in that the 8 years of fertilizer addition before the start of the incubation was not continued during the incubation. Thus, the major effect of the fertilizer should have been as a determinant of the initial soil chemistry,

reflecting fertilizer effects on decomposition, as well as litter inputs over the previous eight years.

In contrast, Mack et al. (2004) found that 20 years of fertilizer addition to a tussock tundra at Toolik Lake caused a 25-30% net loss of C from the entire soil profile relative to controls, despite a greater than 2-fold increase in litter input over the 20 years. This is the opposite of what we found in the incubation of fertilized tussock and intertussock soils. However, our initial sampling of tussock and intertussock soils suggested that both the thickness of the organic mat and its bulk density were lower in fertilized tussock and intertussock soils than in the controls although the differences were not significant. Resampling of the same plots in 2002 (the 13th year of fertilizer treatment) also showed thinner organic mats and lower bulk densities in fertilized tussock and intertussock soils (H. Rueth, unpublished data; M. Sommerkorn and K. Clemmensen, unpublished data).

The fact that tussock and intertussock soils lose C in long term fertilizer experiments (Mack et al. 2004) while incubated soils from the same plots lose C less rapidly than the controls (Schimel and Weintraub 2003, this study), indicates that additional factors must be important in regulation of overall soil C turnover. Perhaps the most important factor is the presence of live vegetation and its inputs of fresh litter and other labile C such as exudates and leachates. Jonasson et al. (2004), for example, showed that plants and their litter have significant effects on microbial C, N and P mass, and on the respiration rate of subarctic heath soils from Abisko, Sweden. In our incubation experiment there were no live plants or fresh litter.

Conclusions and Scaling up

We conclude that in Alaska tundra ecosystems much of the site-to-site variation in C losses from soil organic matter is related to the chemical quality of the organic matter and to

temperature and moisture conditions. The minor effects of long-term fertilizer addition on C loss, under the conditions of this experiment, were probably the result of effects on organic matter quality at the start of the incubation rather than any effect of higher N or P availability during the incubation. The amount of C that can be potentially mineralized in these soils is large, so the rate of C loss is determined more by the relative abundance of substrates of varying quality than by depletion of a small, labile pool. All four C fractions that we studied both lost and gained C, indicating that even the most recalcitrant fractions are at least somewhat mineralized and that microbial products also are added to these pools. The lack of convergence in C chemistry over the course of these long-term incubations is a reflection of the turnover of all four fractions.

As the arctic climate warms in the coming decades and centuries (ACIA 2004), we expect that soil organic matter will lose C at a rate that may continue for many years even without fresh, labile soil C inputs. This C loss will be higher if the climate is drier and/or the soils are better drained. The rates of C loss will also vary among the major ecosystem types, with the lowest loss rates in wet sedge tundra even under warmer and drier conditions. Most of the losses will be as CO₂ although CH₄ losses may also increase under warmer and wetter conditions (Christensen et al. 2003, Callaghan et al. 2004b).

The major remaining uncertainties include: (1) interactions of the C cycle with other elements, particularly N and P; and (2) interactions of relatively old soil organic matter with living plants. Effects of other elements have not been studied in detail in lab experiments on tundra soils, but modeling and incubation studies are consistent with N-limitation (Schimel and Weintraub 2003, Weintraub and Schimel 2003, 2005b), and long-term addition of N and P fertilizer in tussock tundra leads to large overall C losses (Mack et al. 2004). In the present study

the initial N content was not strongly affected by the pre-incubation fertilizer addition; although P content was consistently higher in fertilized soils, it had no clear effect on C losses. Effects of live plants, through root exudates, mycorrhizal associations, and the competitive effects of plants on microbial growth and uptake, can be highly significant (e.g., Loya et al. 2002, Schimel and Bennett 2004, Schmidt et al. 1997, 2002, Jonasson et al. 2004, Olsrud et al. 2004, Pendall et al. 2004). In the present study we isolated soil organic matter from these effects, but isolation and quantification of plant effects on C turnover in soil organic matter is a particularly high priority for future research.

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Table 1. Initial homogenized soil organic matter properties (before incubation). For each soil type, significant differences ($P < 0.05$) between control (C) and fertilized (F) treatments are indicated in **bold**.

Soil type	Treatment	%C		%N		Total P (mg/g)	
		mean	SE	mean	SE	mean	SE
Heath	C	32.08	0.44	1.28	0.03	1.02	0.02
	F	28.8	1.58	1.33	0.07	2.05	0.07
Intertussock	C	31.08	0.54	1.32	0.01	1.20	0.01
	F	28.55	0.08	1.27	0.01	1.63	0.02
Sedge	C	38.38	0.06	2.52	0.01	0.85	0.00
	F	37.89	0.19	2.49	0.01	1.11	0.01
Tussock	C	30.53	0.79	0.92	0.04	0.89	0.03
	F	36.15	0.99	0.99	0.02	1.14	0.00

Table 2. Summary of ANOVA results for soil type and fertilizer effects on initial, homogenized organic matter fraction concentrations (g OM per g bulk soil). Asterisks denote significance at $P < 0.1$ (+), $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***)

Effect	Organic Matter Fraction				Sum of fractions
	NPE	WS	AS	AIS	
Soil type (S)	***	***	***	***	***
Fertilizer (F)	***		***	***	
S x F	***	+	***	***	***

Table 3. Summary of 3-way factorial ANOVA results for effects of fertilizer, temperature, and soil moisture treatments on cumulative CO₂-C loss and total C mass loss in the long-term soil incubation experiment. “C mass loss” is the C loss calculated as the difference in C mass between the start and end of the experiment. Asterisks denote significance at P<0.05 (*), P<0.01 (**), and P<0.001 (***).

Variable	Effect	Soil type			
		Heath	Intertussock	Sedge	Tussock
CO ₂	Fertilizer (F)				***
	Temperature (T)		***	***	***
	Moisture (M)	***	***	***	***
	F x T				
	F x M				***
	T x M		***	*	***
	F x T x M				
C mass loss	Fertilizer (F)		***	**	***
	Temperature (T)	***	**	***	***
	Moisture (M)	***			***
	F x T				
	F x M				
	T x M	*			*
	F x T x M				

Table 4. Summary of soil type-by-soil type ANOVA results for effects of fertilizer, temperature, and soil moisture treatments on the proportional change (increase or decrease) in the four organic matter fractions in the long-term soil incubation experiment. All data were transformed as $\text{LOG}_{10}(100+\%$ change from initial value). NPE=nonpolar extractable; WS=water soluble; AS=acid soluble; AIS=acid insoluble. Asterisks denote significance of main effects and interactions at $P<0.1$ (+), $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$ (***)).

Variable	Effect	Soil type			
		Heath	Intertussock	Sedge	Tussock
NPE	Fertilizer (F)	*	***		***
	Temperature (T)				
	Moisture (M)				
	F x T				
	F x M				
	T x M				
	F x T x M				
WS	Fertilizer (F)	**			
	Temperature (T)				
	Moisture (M)				
	F x T				
	F x M				
	T x M				
	F x T x M				
AS	Fertilizer (F)	***		***	*
	Temperature (T)		*	**	*
	Moisture (M)				*
	F x T				
	F x M				
	T x M				
	F x T x M				
AIS	Fertilizer (F)	**	*	***	
	Temperature (T)	+		+	**
	Moisture (M)		**		***
	F x T				
	F x M				*
	T x M			+	
	F x T x M				

Table 5. Summary of soil type-by-soil type ANOVA results for recovery of ^{13}C as percent of the initial amount of ^{13}C added, for bulk soil and by soil C fraction. Because only the control, unfertilized soils were labeled, there is no test for a fertilizer effect. NPE=nonpolar extractable; WS=water soluble; AS=acid soluble; AIS=acid insoluble. Asterisks denote significance of main effects and interactions at $P<0.1$ (+), $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$ (***).

Variable	Effect	Soil type			
		Heath	Intertussock	Sedge	Tussock
Total ^{13}C	Temperature (T)	**	***	+	**
	Moisture (M)		*		
	T x M				
^{13}C NPE	Temperature (T)		*		
	Moisture (M)	+	*		+
	T x M				
^{13}C WS	Temperature (T)	*	**		
	Moisture (M)	**	**	+	
	T x M	*	+		
^{13}C AS	Temperature (T)	*	**		*
	Moisture (M)	**	*		**
	T x M				
^{13}C AIS	Temperature (T)	**	*		*
	Moisture (M)	***	***		*
	T x M	**			

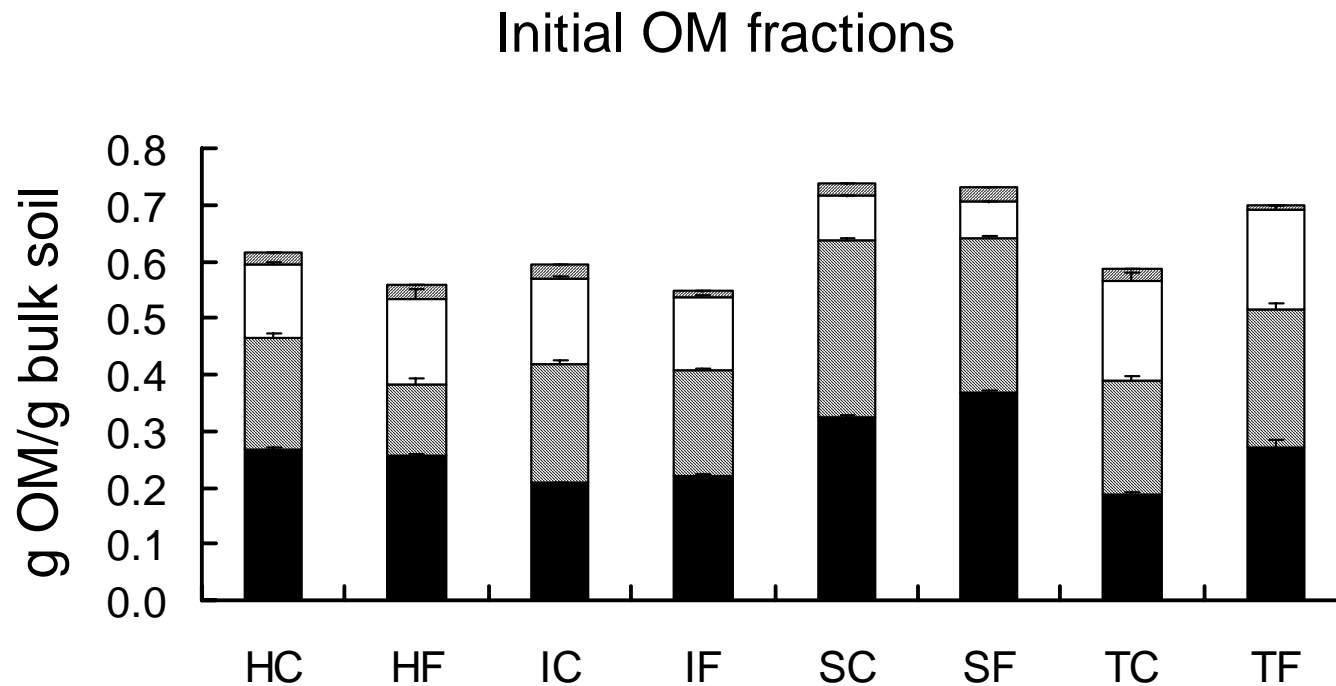


Figure 1. Initial concentrations (g organic matter per g bulk soil) of the four organic matter fractions in homogenized soils used in the incubation experiment. Black segments=acid insoluble (AIS) fraction; lower hatched segments=acid soluble (AS) fraction; clear segments=water soluble (WS) fractions; upper hatched segments = nonpolar extractable (NPE) fraction. Heath, intertussock, sedge, and tussock soil types are indicated by the letters H, I, S, and T, respectively; C indicates control soils and F indicates fertilized soils.

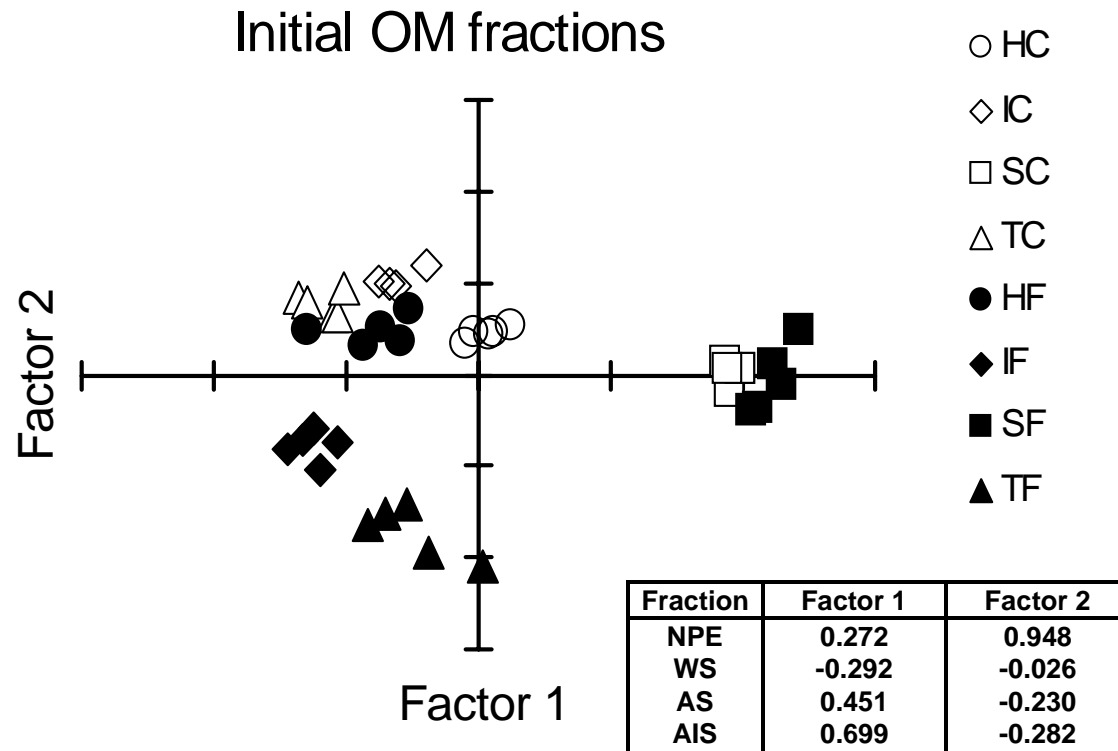


Figure 2. Discriminant analysis of organic matter fraction data in Figure 1. There were 8 groups of 5 replicate samples, each group representing one of the initial 8 soil type x fertilizer combinations. Each sample was characterized by its AIS, AS, WS, and NPE concentrations. The first discriminant function (horizontal axis) accounts for 63% of total variance, and the second discriminant function accounts for an additional 17%. Both axes are highly significant by Wilk's λ criterion ($P < 0.001$), indicating significant differences among groups on both axes. The box at lower right shows canonical discriminant function loadings for each organic matter fraction (i.e., the relative correlations of each fraction with each of the first two discriminant functions). Each point on the graph represents a single sample. HC=heath control; HF=heath fertilized; IC=intertussock control; IF=intertussock fertilized; SC=sedge control; SF=sedge fertilized; TC=tussock control; TF=tussock fertilized.

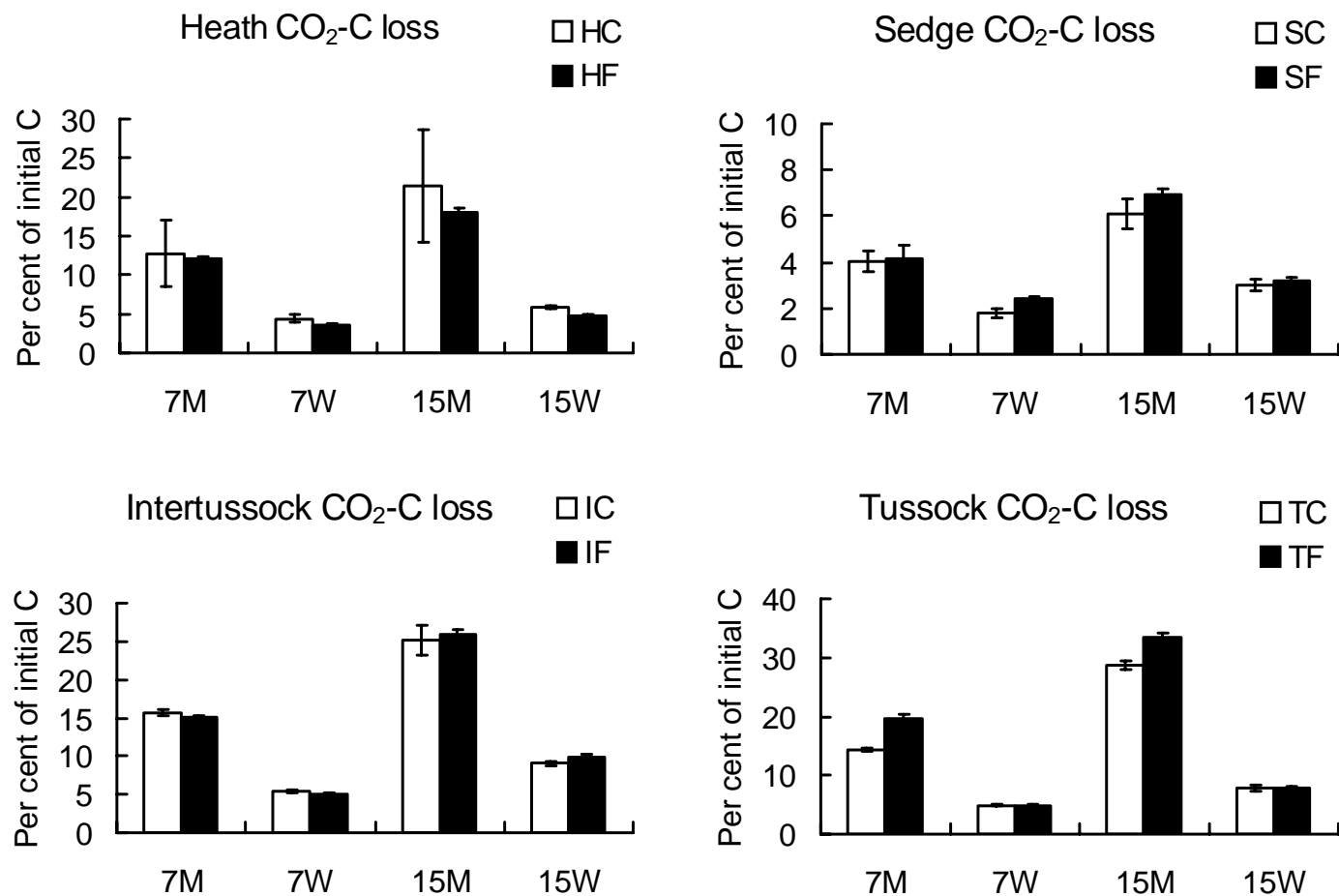


Figure 3. Integrated CO₂-C losses over the duration of the incubation experiment, as percent of the initial C mass. Unfilled bars are control soils; filled bars are fertilized soils. 7M=moist, 7°C; 15M=moist, 15°C; 7W=wet, 7°C; 15W=wet, 15°C.

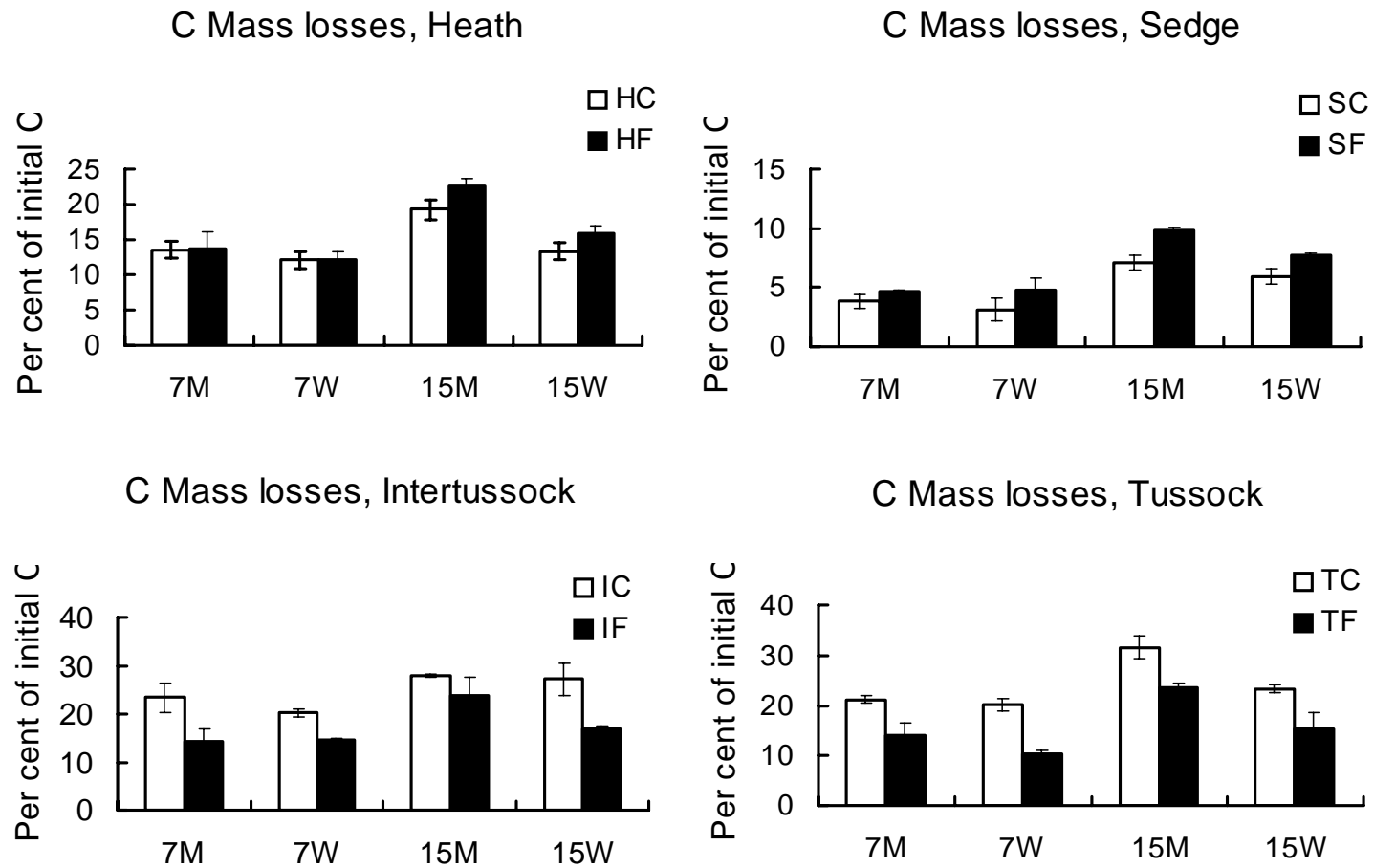


Figure 4. Total C losses as percent of initial C content, calculated for each core as the change in C mass from the start to the end of the experiment. (clear = control, filled = fertilized).

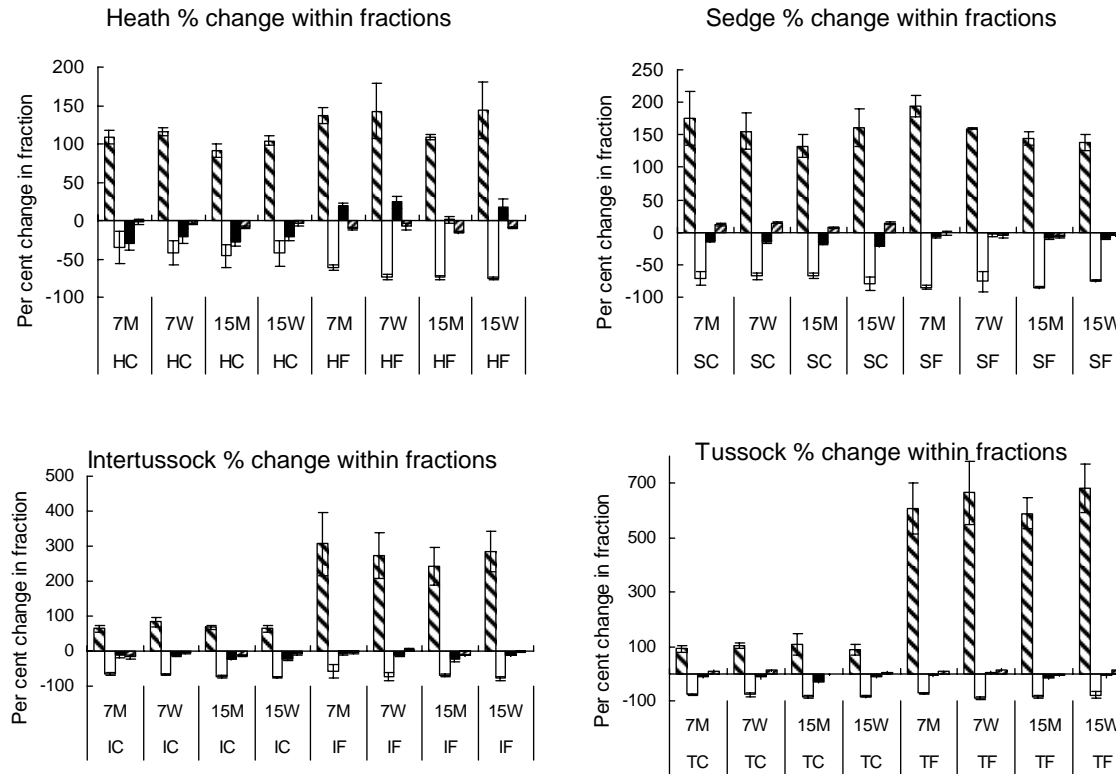


Figure 5. Final organic matter composition in the incubation experiment, expressed for each fraction as the percent change in fraction mass, i.e. $\left(\frac{\text{Final fraction mass} - \text{Initial fraction mass}}{\text{Initial fraction mass}}\right) \times 100$. Bars represent the four organic matter fractions at the end of the incubation experiment, expressed as fractions of the initial amount of organic matter. From left to right within each group, these are: first hatched bars = nonpolar extractable (NPE) fraction; clear segments = water soluble (WS) fraction; black bars = acid soluble (AS) fraction; second hatched bars = acid insoluble (AIS) fraction. Heath, intertussock, sedge, and tussock soil types are indicated by the letters H, I, S, and T, respectively; C indicates control soils and F indicates fertilized soils. 7M=moist, 7°C; 15M=moist, 15°C; 7W=wet, 7°C; 15W=wet, 15°C.

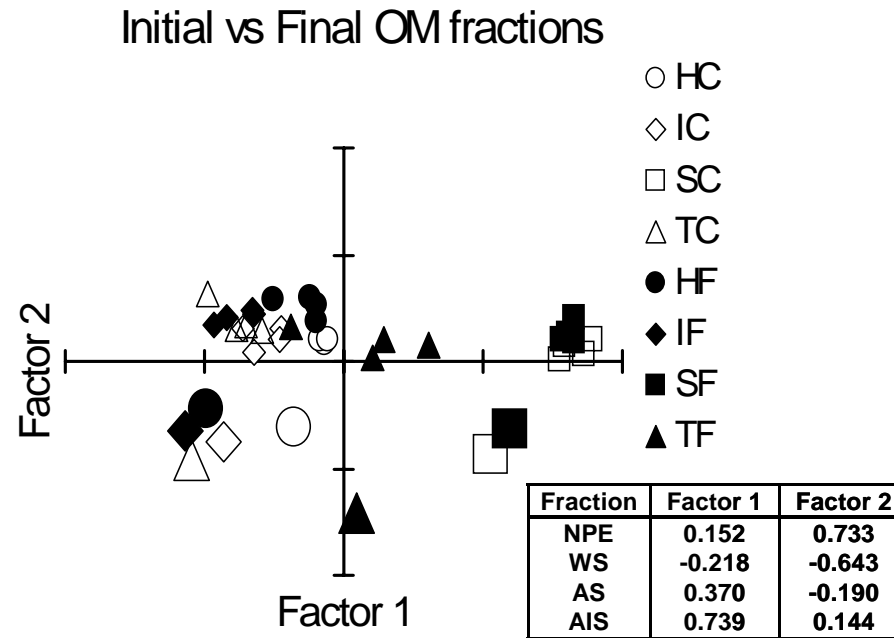


Figure 6. Discriminant analysis of initial and final organic matter fraction data. In this analysis, there were 40 groups including the 8 initial soil type x fertilizer treatment combinations and the 32 final soil type x fertilizer x temperature x moisture treatments. Each sample (2-5 samples per group) was characterized by its AIS, AS, WS, and NPE concentrations, calculated as g organic matter per g bulk soil. Each point on the graph represents the centroid for each group. The 8 large-font data points (all in the lower half of the graph) represent the group centroids for the 8 initial homogenized soils, and the 32 smaller-font data points (all in the upper half) represent the group centroids for the final, post-incubation soils (8 initial soils x 2 temperature x 2 moisture treatments). The first discriminant function (horizontal axis) accounts for 71% of total variance in the data set, and the second discriminant function accounts for an additional 21%. Both axes are highly significant by Wilk's λ criterion ($P < 0.001$), indicating significant differences among groups on both axes. HC=heath control; HF=heath fertilized; IC=intertussock control; IF=intertussock fertilized; SC=sedge control; SF=sedge fertilized; TC=tussock control; TF=tussock fertilized.

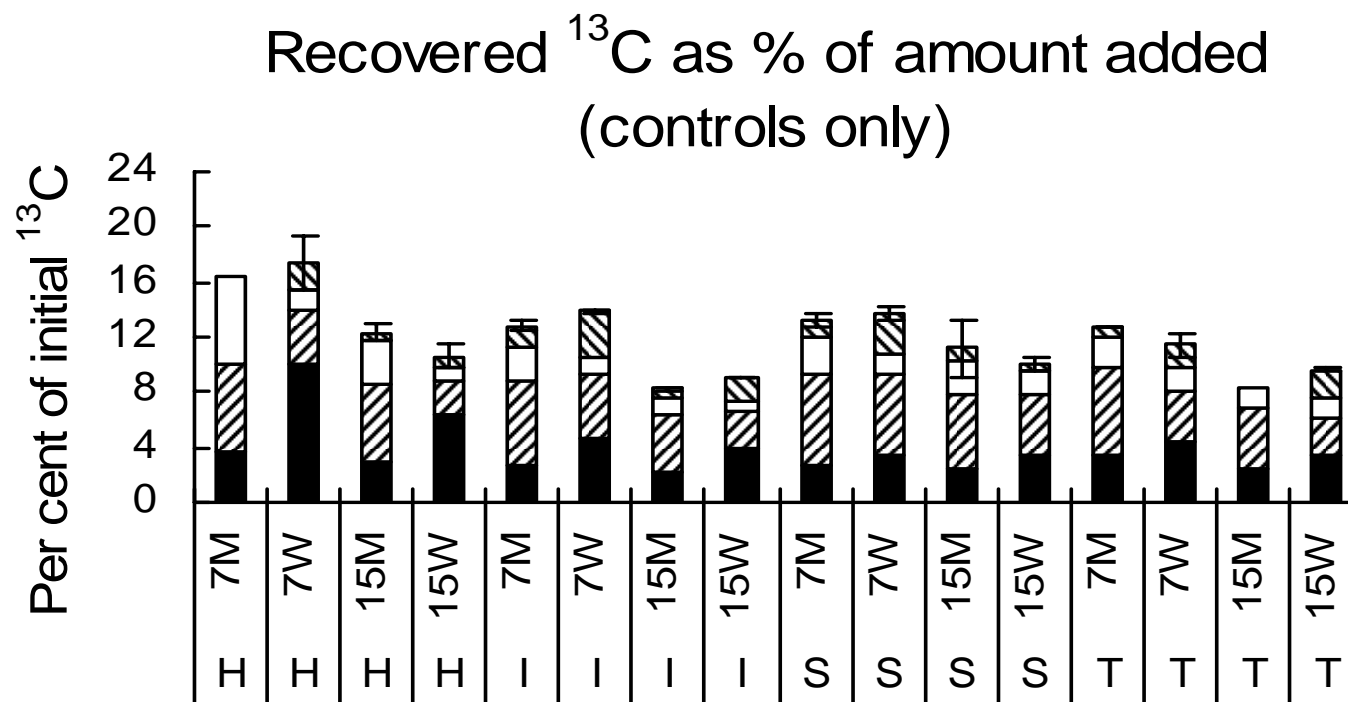


Figure 7. Recovery of ^{13}C at the end of the incubation experiment as a percentage of the total amount added to each core at the start of the experiment. Black segments=acid-insoluble (AIS) fraction; lower hatched segments=acid-soluble (AS) fraction; clear segments=water-soluble (WS) fractions; upper hatched segments = nonpolar extractable (NPE) fraction. Heath, intertussock, sedge, and tussock soil types are indicated by the letters H, I, S, and T, respectively. Only the control soils received the ^{13}C -labeled glucose. 7M=moist, 7°C; 15M=moist, 15°C; 7W=wet, 7°C; 15W=wet, 15°C. Error bars indicate standard error of the mean for the sum of the ^{13}C in all four C fractions.