DISSERTATION

SOIL ORGANIC MATTER AND AGGREGATE DYNAMICS IN AN ARCTIC ECOSYSTEM

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ABSTRACT

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Warming has been linked to changes in Arctic soil carbon cycling. Cold temperatures and anoxic conditions in the Arctic diminish microbial activity. As a result mineralization rates are low and the system is nitrogen-limited, further reducing biological activity. Reducing this constraint on nutrient availability has resulted in a vegetation shift and loss of soil carbon; however, the mechanisms behind soil carbon loss are not well understood. The focus of this study was on the active mineral layer directly below the organic horizon.

Soils were collected during the 2007 growing season from a long-term nutrient addition experiment in which soils had been fertilized with additional N and P since 1996 and 1989 at the Arctic LTER site at Toolik Lake, on the Alaskan North Slope. Roots were separated from the soil to estimate biomass. Soils were separated into four size classes of water-stable aggregates (Large and small macroaggregates, microaggregates, and silt+clay). Small macroaggregates were separated into three sub-fractions (coarse particulate organic matter (POM), occluded microaggregates, and silt+clay). Density floatation was used to separate light fraction (LF) organic matter

from heavy fraction in small macroaggregates and microaggregates. Intra-aggregate POM (iPOM) content was determined in small macroaggregates and microaggregates. Differences in aggregate size distribution, C and N allocation, and C:N in each fraction were analyzed.

Small Macroaggregates were the dominant aggregate fraction in all treatments. Mid-season declines in large macroaggregate abundance from soils with nutrient addition differed statistically from the control, though both comprised <10% of the whole soil. The ratio of free:occluded microaggregates rose over the growing season, which indicated that microaggregates occluded within small macroaggregates were released upon macroaggregate disruption. Occluded microaggregates tended to possess higher carbon and nitrogen contents than free microaggregates due to increased physical protection within the macroaggregate. As a result, the ratio of free:occluded microaggregate C:N declined over the growing season, possibly due to N-rich, formerly occluded microaggregates entering the free microaggregate pool. Nutrient addition resulted in changes in C allocation in the small aggregate LF and microaggregate iPOM to an increasingly large amount over the growing season. Nitrogen allocation responded in a similar manner, resulting in a lower C:N in the LF of soils under nutrient addition since 1989. Nutrient addition resulted in an increase in root biomass by the middle of the growing season; however by the final sampling date, root biomass declined.

Nutrient addition affected aggregate size class distribution only in mid-June, which indicated that this is a dynamic period of aggregate formation and may be dependent on the microbial community and N availability. Macroaggregate turnover, as evidenced by free:occluded microaggregate abundance, occurred earlier in the growing

season in soils with nutrient addition than the control. As a result, SOM formerly occluded within macroaggregates may be increasingly susceptible to decomposition by the microbial community over the growing season. The re-allocation of SOM from physically protected aggregates to light fraction with nutrient addition may result in shifts in SOM stability in these soils. The observed increases in the proportion of soil carbon as light fraction and iPOM with nutrient addition indicate a shift towards an increase in POM fractions that tend to be labile, potentially mineralizable sources of organic matter. The balance between the rates of organic matter input and decomposition may favor decomposition, resulting in a short-term loss of carbon in Arctic soil. Carbon content may stabilize in the future as its remaining stocks become increasingly processed by the microbial community. These results highlight the importance of multiple sample collection dates, which are necessary if we are to improve our understanding of factors driving SOM stabilization in Arctic soils.

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CHAPTER ONE

LITERATURE REVIEW AND BACKGROUND

Section 1.1. Introduction: Changes in Low arctic systems

Arctic ecosystems contain an estimated 14% of the global terrestrial carbon pool (Post *et al.* 1982), stored in soil organic matter (SOM). Cold temperatures and anoxic conditions in the Arctic inhibit microbial activity, lowering decomposition rates and thereby stabilizing soil organic matter (Hobbie *et al.* 2000), which is formed from decomposing plant material, animal carcasses and excrement, and microbes (Schreiner and Shorey 1911; Miller and Gardiner 1998). Under these conditions, mineralization rates are low relative to plant demand, resulting in a nitrogen-limited-system, further reducing biological activity. Until recently, climatic conditions had remained relatively stable since the beginning of the Holocene epoch 10,000 years ago, resulting in a net carbon sink (Greene *et al.* 2008). Warming trends in the Arctic appear to have altered this climatic constraint on soil biological activity, leading to increased decomposition and soil CO_2 efflux (Doles 2000; Oechel *et al.* 2000; Hobbie *et al.* 2002; Mack *et al.* 2004).

Arctic ecosystems are being intensely studied with regard to nutrient cycling because observed local-level to ecosystem-level changes in the system, particularly vegetation and carbon storage dynamics, may be accurate early indicators of broader-scale changes in global vegetation and carbon cycling dynamics to come (Shaver *et al*. 1992). Rising temperature in the Arctic has resulted in longer growing seasons due to earlier thaw and later freeze, which impacts vegetation composition (Stow *et al.* 2004). Furthermore, long-term nutrient addition experiments (1982-present) have demonstrated a shift from tussock tundra dominated by *Eriophorum vaginatum* to shrub tundra dominated by the dwarf arctic birch, *Betula nana* (Chapin *et al.* 1995; Shaver *et al.* 2001).

Preliminary fieldwork was conducted (a detailed description of which is provided below) at a long-term nutrient addition site located at the Arctic Long Term Ecological Research (LTER) site at Toolik Lake, Alaska. The results from this work demonstrated the presence of soil macroaggregates in arctic soils and provided evidence that soil structure (aggregate formation) is positively linked to carbon storage in arctic soils and is affected by change (nutrient addition). This preliminary work led to several questions (details below) regarding the location and stability of SOM within the soil matrix:

1. What linkages exist between soil C and macroaggregate sub-structure elements in arctic soils?
2. What are the temporal dynamics of aggregates in arctic soils over the growing season?
3. Is SOM more stable within aggregates compared to non-aggregate-associated light fraction?
4. What are the relationships between light fraction and live root biomass dynamics?

Based on the above questions, the following hypotheses were developed, and a more detailed description will be provided below:

H1: Aggregate size distribution in arctic tundra is dynamic under natural conditions and responds to changes in organic matter inputs, microbial activity, and temperature.

H2: Aggregate carbon and nitrogen content in arctic tundra are dynamic under natural conditions and respond to changes in organic matter inputs, microbial activity, aggregate size distribution, and temperature.

H3: Macroaggregate substructure size distribution and C:N in arctic tundra are dynamic under natural conditions and respond to changes in organic matter inputs, microbial activity, and soil macroaggregate turnover.

H4: Particulate organic matter carbon content, nitrogen content, and location within the soil matrix as either light fraction or iPOM in arctic tundra are dynamic under natural conditions and respond to changes in organic matter inputs, microbial activity, soil aggregate turnover, and temperature.

Subsequent sections in this chapter will provide the rationale behind the above hypotheses. The following two subsections introduce two topics related to arctic change: (1) the impact of rising temperature in the Arctic and (2) the impact of shifts in vegetation cover and composition.

Section 1.1 Subsection 1: Impact of rising temperature

Arctic ecosystems have warmed significantly over the past 30 years (Oechel *et al.* 2000; Serreze *et al.* 2000). An increase in CO_2 and CH_4 production with increased SOM decomposition may result in a positive feedback to increased temperatures (White *et al.* 2004). Over time, a positive respiration feedback to temperature may result in carbon loss from arctic soils. Carbon in the organic horizon would be lost through increased decomposition, while increased temperature would lead to a widening of the active mineral layer through permafrost thawing (Schuur *et al.* 2009), which would expose organic matter to decomposition and resulting CO_2 efflux.

Increasing temperature in the Arctic may result in longer growing seasons due to earlier thaw and later freeze, increased precipitation, and increased nutrient availability (reviewed in Stow *et al.* 2004; Tape *et al*. 2006). These changes have the potential to impact vegetation composition. On a short-term scale, carbon loss is predicted to occur from arctic soils, but shrub expansion and subsequent shift of biomass to aboveground woody tissue may stabilize arctic terrestrial carbon, though not in the soil (Oechel *et al*. 1993). While increased decomposition would result in carbon loss, the remaining carbon would be more processed and therefore more chemically stable than current carbon stocks. An increase of shrub abundance over the past 50 years has been documented (Sturm *et al.* 2001).

Arctic soils are nutrient-limited due to unavailable organic matter in the permafrost layer (Hobbie and Chapin 1998). As this layer thaws due to increasing temperature, more labile nutrients will become available to the microbial community, which can potentially result in a greater efflux of CO_2 from these soils (Hobbie *et al*. 2002; Grogan and Chapin 2000). Increases in nitrogen availability may alter SOM turnover by altering decomposition and plant growth rates (Neff *et al*. 2002).

Microbial activity and the nutrient cycles they mediate may also be affected by increased temperatures (Oelbermann *et al*. 2008). Microbial respiration rapidly increases in response to increased temperature. Uchida *et al*. (2010) measured a 20% increase in microbial respiration within one hour of increasing soil temperature by 2^{o}C from a base temperature of 9^{o}C on soils from a New Zealand grassland. However, higher temperature increases of 24^{o}C reduced activity. Nadelhoffer *et al*. (1991) conducted a series of incubations of arctic soils and found that carbon and nitrogen mineralization rates were not affected by temperatures below 9^{o}C, but doubled when the temperature was increased to 15^{o}C. Increases in temperature remove constraints on microbial enzyme activity and on microbial metabolism in general, thereby increasing decomposition rates; however, constraints on nutrient availability may still remain (Wallenstein *et al*. 2009).

Microbially-produced enzymes that metabolize structurally-complex molecules generally have higher Q_{10} values for carbon mineralization, as evidenced by low respiration rates with low temperature (Mikan *et al*. 2002). Decomposition of relatively stable organic compounds is more temperature sensitive than labile compounds, meaning as temperatures rise, more resistant pools of organic matter will be relatively more sensitive than labile pools, if Q_{10} is higher for resistant compounds (Kirschbaum 1995, 2006; Koch *et al*. 2007; Conant *et al*. 2008). Reducing at least one constraint (temperature) on the decomposition of organic matter with intermediate turnover rates could potentially result in carbon loss.

Direct N+P additions to tundra have resulted in carbon losses from soil. Mack *et al.* (2004) reported a net carbon loss in soils after nearly 20 years of nutrient addition, including a 50% decrease in root biomass. This carbon loss occurred through the whole soil profile, but was greatest in mineral soils underlying a thick organic layer, which is of particular interest because carbon stored in this layer had been sequestered in the past, but as nutrient availability became less of a limiting factor through warming, the pool was depleted. We do not know whether this observed carbon loss will continue or if it is a component of a shift to an altered steady state, nor do we know if the carbon loss observed by Mack *et al*. (2004) occurred at a steady rate or if there were changes in the rate of carbon loss. A large fraction of the global soil carbon pool is found within Arctic systems; therefore, an increased rate of carbon dioxide release from tundra soils will have a potentially major impact on atmospheric greenhouse gas concentration (Melillo *et al*. 1990).

Section 1.1 Subsection 2: Impact of Changes in Vegetation

Much of the observed change in arctic tundra vegetation has been focused on conversion from tussock tundra to shrub tundra. This change occurs when the vegetation transition zone is correlated with climatic factors, including summer temperature and winter snow conditions, and dominant species have properties that allow them to increase in abundance following a climate shift (reviewed in Epstein *et al.* 2004).

Changes in the vegetation composition, including a shift from tussock to shrub tundra in response to nutrient addition, has been demonstrated in the Arctic (Shaver *et al.* 2001; Sturm *et al.* 2001; Tape *et al.* 2006). Chapin *et al*. (1995) found that vegetation biomass increased after three years of nutrient addition, but composition did not change; however, after nine years, *Betula nana* dominated plots that had nutrient addition. *Betula* dominance increased from 70% after nine years to 90% of total plant cover after 15 years of nutrient addition (Shaver *et al.* 2001). This dominance was not based on recruitment, but on the increased growth rate of individuals already present (Bret-Harte *et al.* 2002). As a result the experimental plots shifted from being tussock tundra to an intermediary form between tussock and shrub tundra. Previous nutrient addition studies have shown that in *Betula*, stem length increases for the first few years, then declines as branching increases in later years (Bret-Harte *et al.* 2002), which results in a plant structure that differs from those measured under normal conditions.

A shift from tussock to shrub tundra could have a potentially far-reaching impact. The organic layer of soil is thinner under shrub tundra than tussock tundra. Soil carbon is also less in shrub tundra, and winter temperatures are warmer under shrubs than tussocks. Also, shrub branches protruding above the snow layer help to conduct heat to the immediate area around the shrub, leading to accelerated snowmelt and a positive feedback to warming (Chapin *et al*. 2005). These factors can result in increased CO_2 efflux from these soils (Epstein *et al.* 2004).

Under control conditions, *Betula* produces mostly short shoots. Bret-Hart *et al.* (2001) reported that nutrient addition resulted in an increase in long-shoot production in *Betula,* which is of importance because long shoots are able to produce branches, whereas short shoots are not. The extensive branching of *Betula* with nutrient addition affected canopy height and structure, and its leaf area index tripled with nutrient addition (Bret-Harte *et al.* 2001). However, a warming treatment using greenhouses had little effect on long shoot production, except when greenhouses were combined with nutrient addition. These results demonstrated developmental plasticity in response to increased nutrient availability in that additional apical meristems were recruited for shoot elongation. A greater number of meristems has allowed *Betula* to greatly increase its branching which has enabled it become dominant over the other vegetation types in response to nutrient addition, which in turn may affect ecosystem-scale processes (Bret-Harte *et al.* 2001).

Vegetation shifts may result in a reallocation of carbon from belowground to woody structures aboveground (Chapin *et al.* 1995). This alone does not explain the soil carbon loss reported by Mack *et al*. (2004) because fresh organic matter inputs, consisting of leaf litter and root exudates still enter the SOM pool. Sullivan *et al*. (2007) found that long-term nutrient addition led to a shift in root biomass production and distribution. *Eriophorum*, the tussock graminoid, has deep annual roots whereas *Betula*, the shrub whose growth is enhanced by nutrient addition, has long-lived roots that are shallower than the *Eriophorum* roots. This shift in root dynamics has led to a loss of carbon at lower depths.

Soil structure is a central component of conceptual models that effectively explain relationships between soil structure and SOM dynamics within temperate systems, particularly in cultivated systems and temperate grasslands (Elliott 1986, Six *et al.* 1998, 1999). These conceptual models could possibly be applied to arctic soils, but would need to be modified in order to account for differences in arctic vs. temperate soils. For instance, in tussock tundra the active mineral soil layer is the interface between organic soil and permafrost soil, and is influenced by both permafrost below and organic soil above it. Interactions between the organic and mineral soil horizons occur within the active mineral layer, including organic matter cycling and belowground foodweb activity. These interactions may be dependent on physical stabilization of SOM through the biologically-mediated formation of water stable soil aggregates.

In the following sections, descriptions of soil, soil organic matter, and soil aggregate dynamics are provided. An attempt is made to link the concepts of soil carbon storage and soil aggregate dynamics with the previously-described changes in the Arctic. Results from the 2006 field season are provided, and the objectives of the 2007 field season are introduced, accompanied by four conceptual hypotheses that form the basis of this dissertation.

Section 1.2. Soil, Soil Organic Matter, and Soil Aggregates

In this section, concepts related to soil, soil organic matter, and soil aggregate dynamics that are relevant to my research are introduced. This section is separated into six subheadings, starting with a general description of soil and soil formation factors, followed by discussions of soil organic matter, soil aggregates, and linkages between aggregates and soil carbon storage, and ending with a description of aggregate substructure and light fraction organic matter. The objective in this section is to increasingly narrow the scope of interest down to the light fraction organic matter while keeping the overall concept of the dynamic role of soil in terrestrial carbon storage in context.

Section 1.2 Subsection 1: Soil

Soil is comprised of particles that may include sand, silt, clay, and organic matter as well as pore space between the particles which is filled with gases or water. Soil is discernable from the parent material from which it originated in that it has undergone physical and chemical weathering of primary and secondary minerals (Birkeland 1999; Soil Survey Staff 1999). Jenny (1994, 1st edition published in 1941) provided a fundamental equation of soil formation, $s = f(cl, o, r, p, t)$, where the five independent factors of soil formation are climate (cl), organisms (o), topography (r), parent material (p), and time (t). These five factors interact with each other to form soil. In the next paragraph, a brief description of arctic soils with respect to the previously mentioned soil forming factors (Jenny 1994; Miller and Gardiner 1998; Birkeland 1999) is provided.

Soils may take hundreds to thousands of years to develop, and over that time, their characteristics change due to, for example, parent material weathering and organic matter accumulation. On the other hand, soils may be degraded, for example, through thermokarst formation due to thawing permafrost, on a much shorter time scale (Osterkamp and Romanovsky 1999).

Soils in Arctic Alaska are underlain by continuous permafrost, and vary depending on vegetation type, parent material age, and topography. Wet sedge tundra is most commonly found on the arctic coastal plain and occurs on flat, low-lying areas. The two most abundant tundra types, moist acidic and non-acidic tundra, are generally found in upland areas with gentle hillslopes (Shaver and Chapin 1991). The direction a slope faces also influences soil formation; south- and west-facing slopes receive more radiation in the Northern Hemisphere than north- and east-facing ones, which may affect productivity.

Soils in Arctic Alaska are largely formed from glacial till parent material, and the age of the soil is dependent on how long the area has been de-glaciated. Moist non-acidic tundra occurs on relatively young soils that were deglaciated between 11,500-25,000 years ago (Hamilton 1986). Because they are not as weathered, moist non-acidic tundra soils have more base cations than moist acidic tundra. Moist acidic tundra occurs on older soils that have undergone more weathering and paludification than non-acidic tundra (Walker *et al*. 1994), and may have been deglaciated longer than 100,000 years ago (Hamilton 1986).

Section 1.2 Subsection 2: Soil Organic Matter

According to Jenny (1994), soil may be treated as an open system with components entering or leaving the soil, including SOM. Changes in the dynamics of the five soil forming factors can influence the accumulation or release of SOM carbon, which may range from 0.08% in Antarctic dry valley soils to 50% in peat soils (Freckman and Virginia 1997; Chiou *et al*. 2000). Soil organic matter originates primarily from plant litter and microbial biomass and consists of many different compounds with varying structure, content, and recalcitrance. According to Kögel-Knabner (2002), who reviewed the components of SOM, initial plant inputs may include aboveground plant material such as branches, leaves, and fruits, while belowground plant inputs include roots and root exudates. Plant-derived compounds include cellulose and lignin, both of which decompose slowly (particularly lignin), as well as soluble materials, lipids, and other labile compounds. Proteins, released by both plants and microbes, are a nitrogen-rich source for organic matter that can be easily degraded if not protected by the soil matrix. Microbial contributions to the SOM pool include amino sugars, extra-cellular polysaccharides, and biomass – all of which are easily degraded when they are not mineral-associated (Kögel and Bochter 1985; Kögel-Knabner 2002). These organic matter pools are not stable, but are dynamic and undergo transformations, including humification, which is the formation of increasingly complex and recalcitrant compounds (Zech *et al*. 1997).

In the following sections, relationships between SOM and soil aggregates are described. The relationship between the two is mutualistic – aggregates formed through biotic means require organic matter as a nucleation site while at the same time, organic matter gains physical protection from decomposition by being occluded within the aggregate. As described in an earlier section, Arctic SOM is stabilized by cold temperatures and an anoxic soil environment. If warming results in a reduction of these two restrictions, physical protection of SOM through stabilization in aggregates may moderate organic matter decomposition rates and subsequent SOM loss in the Arctic if aggregation increases due to climate change and if aggregate-associated SOM is less sensitive to climate change than non-aggregate-associated SOM.

Section 1.2 Subsection 3: General description of Soil Aggregates

Soil aggregates are the basic units that define soil structure and are comprised of sand, silt, clay, and organic matter bound together by organic and inorganic binding agents (Tisdall and Oades, 1982; Tisdall, 1994). Aggregates have typically been categorized into two major size classes: macroaggregates (>250μm) and microaggregates (53-250μm) (Tisdall and Oades 1982; Tisdall 1994). Aggregates provide a means to stabilize SOM through physical protection (Buyanovsky *et al*. 1994).

Soil aggregates are dynamic entities within the soil matrix, with turnover rates dependent on several factors, including the microbial community, SOM quality, and the soil environment, including clay content, wet/dry cycles, and freeze/thaw cycles (reviewed in Six *et al.* 2004). The process of aggregate formation is initiated when microbes begin to decompose a piece of particulate organic matter (POM) or dissolved organic matter (DOM) that has been adsorbed onto clay surfaces (Reviewed in Guggenberger and

Kaiser 2003). The extracellular polysaccharides excreted by the microbes using the POM as a substrate, cause clays and other soil particles to stick around the POM. This eventually results in the creation of an aggregate. Particulate organic matter is a SOM fraction that can be isolated from mineral-associated organic matter through density separation (Golchin *et al*. 1995) and is highly susceptible to degradation following soil disturbance, and is partially protected within aggregates from decomposition (Cambardella and Elliott 1992). When macroaggregates are disrupted, there is a loss of SOM due to organic matter decomposition (Elliott 1986).

Once SOM is encrusted with clay and becomes part of an aggregate, the rate of decomposition of that material is reduced as oxygen becomes limiting to the microbial community (Oades 1984). As organic matter held within macroaggregates is decomposed, microbially-produced mucilages combine with clay to initiate microaggregate formation (Oades 1984; Six *et al*. 1998). Microaggregates are stabilized by processed organic matter and inorganic binding agents (Tisdall 1994; Molope *et al*. 1987; Beare *et al*. 1997). Hyphal entanglement holds macroaggregates together, but does not provide the structural stability found in microaggregates (Waters and Oades 1991; Beare *et al*. 1994). If aggregate disruption rates exceed rates of aggregate formation (e.g., with soil tillage), then soil organic matter is less likely to be protected within an aggregate than it would be in less-disturbed systems (Elliott 1986; Six *et al*. 1998, 1999). This relationship between aggregate formation and carbon storage will be described in subsequent sections.

<u>Section 1.2 Subsection 4: Role of Aggregates in Soil Carbon Storage and Stabilization</u>

The soil matrix consists of several microenvironments characterized by changes in the microbial community, soil texture, oxygen availability, and SOM content (Ladd *et al*. 1996). Soil organic matter can be stabilized (protected from decomposition) chemically through associations with silt and clay (Feng and Simpson 2008), biochemically through the formation of recalcitrant compounds (e.g., humus), or physically through soil aggregation (Six *et al*. 2002). These factors and processes interact with one another to regulate feedbacks between fresh organic matter inputs and decomposition, including nutrient availability. Aggregates limit the release of nutrient-rich OM, which affects decomposition as well as inputs by regulating plant nutrient uptake. Slow decomposition rates relative to organic matter inputs results in a net gain of carbon, while increased decomposition results in carbon loss through CO_2 efflux.

Soil physical processes, in particular the stabilization of soil carbon through the formation and stabilization of water-stable aggregates provide a mechanism for carbon stabilization and storage in soils (Oades 1984; Six *et al*. 1999; Six *et al*. 2000; Plante and McGill 2002). In cultivated systems, annual disturbance through tillage tends to increase aggregate turnover (Six *et al*. 1998), which has been linked to carbon loss in those systems. Elliott (1986) observed a reduction in macroaggregate formation with cultivation, which was coupled with observations of increased carbon concentration as well as greater amount of new and more labile carbon in macroaggregates vs. microaggregates.

Six *et al*. (1999, 2000) proposed a model of aggregate turnover in which increases in physical disturbance led to a breakup of macroaggregates, which exposed aggregate-protected particulate organic matter (iPOM) fractions to the microbial community. Soil organic matter decomposition rates subsequently increased. Increased physical disturbance led to an increase in macroaggregate turnover. This resulted in a release of iPOM held within the macroaggregate as well as occluded microaggregates. Additionally, by increasing macroaggregate turnover rates, disturbance inhibits microaggregate formation around newly incorporated iPOM within the macroaggregate structure. As a result, there is an increase in carbon-depleted microaggregates in response to increased macroaggregate turnover.

Section 1.2 Subsection 5: Aggregate sub-structure

Aggregate sub-structure refers to the components that comprise an aggregate, including POM, silt+clay, and (in the case of macroaggregates) microaggregates. For example, macroaggregates can be fractionated into their sub-structural elements, including intra-aggregate-particulate organic matter (iPOM), macroaggregate-derived microaggregates, and silt+clay fraction (Six *et al*. 1998, see Fig. 1). Particulate organic matter can be separated from mineral-associated organic matter (Theodorou 1990), and may either be associated with aggregates, or may be free from aggregate association (Six *et al*. 1998). Free POM is referred to as the Light Fraction (LF), and can be separated from the Heavy Fraction (HF), which includes aggregates, and iPOM within the aggregates, through density flotation.

Aggregate formation is not entirely a hierarchical process wherein large aggregates are formed from smaller ones. Rather, iPOM contained within a macroaggregate can become a location of microaggregate formation within the macroaggregate (Oades 1984; Golchin *et al*. 1994; Six *et al*. 1998; Gale *et al*. 2000). When the macroaggregate is disrupted, the microaggregate, which is more stable than the macroaggregate, tends to stay intact longer, thereby providing a physical means of carbon stabilization (Oades 1984; Angers *et al*. 1997). However, organic matter existing as iPOM within the macroaggregate, and not stabilized in a microaggregate within the macroaggregate, would be susceptible to decomposition upon macroaggregate disruption (Denef *et al*. 2001). Six *et al*. (2000) have shown that microaggregates contained within macroaggregates are an important component of soil carbon storage, containing a large proportion of aggregate carbon that is susceptible to degradation if exposed to the decomposer community.

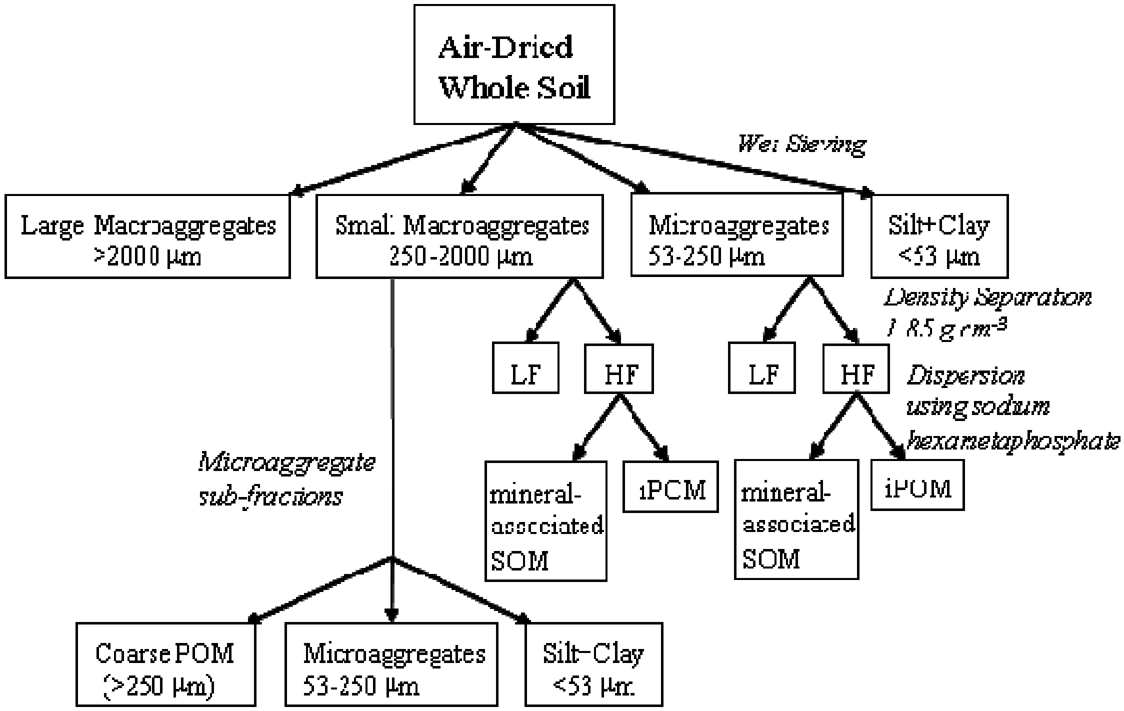

Figure 1.1. Soil fractionation diagram based on methods of Six *et al.* (1999). LF = Light Fraction, HF = Heavy Fraction, POM = Particulate Organic Matter, iPOM = intra-aggregate Particulate Organic Matter.

Natural and anthropogenic disturbances to soils either disrupt the aggregated structure of soils by breaking aggregates down to their sub-structural components, or, change conditions in ways that accelerate the decomposition of more labile organic components. In a grassland system, Six *et al.* (1998) found that a loss of free, non-occluded organic matter (light fraction) made up 42% of the carbon loss attributed to cultivation. In addition, differences in cultivation intensity (no-till vs. conventional till) resulted in a loss of fine inter-particulate organic matter (iPOM) with conventional tillage. Fine iPOM is contained within microaggregates. These results indicate that a feedback loop exists between SOM and aggregate formation. Aggregates protect SOM, but organic matter is also necessary for aggregate formation.

Section 1.2 Subsection 6: Light Fraction organic matter

The light fraction is comprised largely of roots and other plant debris, hyphae, and charcoal (Spycher *et al*. 1983). It has a C:N lower than roots, but greater than mineral-associated organic matter, which indicates that some decomposition has occurred, but it is still relatively labile (Molloy and Speir 1977; Molloy *et al*. 1977; Sollins *et al*. 1984; Theodorou 1990). Light fraction turnover time is slower than fresh litter, but faster than mineral-associated organic matter (Christensen 2001).

The pool size, along with carbon and nitrogen dynamics of the light fraction, are indicators of labile SOM turnover dynamics (Janzen *et al*. 1992). Soil organic matter fractionation has been used to estimate the stability of carbon pools in grassland and cultivated soils. In native sod, Cambardella and Elliott (1992) found that mineral associated carbon constituted 60% of soil organic carbon, while the POM fraction made up the remaining 40%. With increasing cultivation intensity, the proportion of carbon associated with the mineral fraction increased while the POM fraction decreased, along with total carbon. These results suggest that POM-C is the carbon fraction lost with disturbance through cultivation and is an intermediate turnover fraction. Dalal and Mayer (1986) observed an organic carbon loss in light fraction that was 2-11 times greater than HF following cultivation; this loss may have been due to a lack of physical protection of LF carbon.

Section 1.3. Arctic Linkages to Soil Organic Matter dynamics

Long-term nutrient addition experiments at Toolik Lake, Alaska have provided a means by which increases in nutrient availability due to warming are simulated (Shaver *et al.* 2001). These long-term experiments have several components, including type of tundra (moist acidic, moist non-acidic, dry heath, and wet sedge), warming inside greenhouses, grazing exclusion, and time under nutrient addition, with the earliest experiments being started in 1981, and the most recent in 2006 (Chapin and Shaver 1988; Shaver and Chapin 1991; Chapin *et al*. 1995; Hobbie *et al*. 2005). These types of studies have yielded two important results. First, arctic soils are generally nutrient limited, with additions of N and P inducing increased activities of soil biota (Doles 2000) and significant shifts in plant communities from tussock to shrub dominance in response to added N+P in moist acidic tundra (Hobbie and Chapin 1998). Second, 20 years of nutrient addition have led to a decrease in soil carbon (Mack *et al.* 2004). Additionally, shifts in microbial activity have been described in response to changes in arctic nutrient addition and vegetation cover (Doles 2000; Clemmensen *et al*. 2006; Wallenstein *et al*. 2009; Gough *et al*. in preparation; Moore *et al*. in preparation). What is missing is an understanding of the physical mechanisms of carbon storage at play.

Shifts in nutrient availability, vegetation, and the soil microbial community all interact with one another in affecting the decomposition of organic substrates and stabilization of SOM in mineral soil by promoting and protecting soil aggregates (Six *et al*. 1999; Six *et al*. 2000; Plante and McGill 2002). At the same time, the formation and stabilization of the aggregates that protect SOM are also mediated by nutrient availability, vegetation input, and microbial activity (Six *et al*. 2000; Six *et al*. 2004).

<u>Section 1.3 Subsection 1:Role of disturbance</u>

Six *et al*. (1999) developed a conceptual model of soil aggregate temporal dynamics that showed that reduced physical disturbance resulted in greater aggregate stability over time. The model was based on studies of grassland and agricultural soil responses to different forms of tillage and management practices. For example, agricultural soils under conventional tillage are routinely disturbed through regular cultivation, resulting in significant losses of SOM in a few decades, while those under less intrusive no-till or minimum tillage management are less disturbed and retain more of their original SOM. This effect has been documented by Elliott (1986), who demonstrated that physical disturbance results in carbon loss due to aggregate disruption when compared to undisturbed (native grassland) and relatively undisturbed (no-till) soils. Six *et al*. (1998) measured a 30% loss of POM between conventionally-tilled and no-till soils, though the no-till POM content was only 50% of native grassland soil.

Tussock tundra soils, on the other hand, are disturbed though cryoturbation, which results in annual physical disruption through organic and mineral soil mixing along with destroying plant roots through freezing and thawing (Benninghoff 1952; Bockheim *et al*. 1998). Cryoturbation, coupled with cold temperatures, water saturated (ice) conditions, and the resulting low microbial and invertebrate activity leads to an accumulation of SOM (Michaelson *et al*. 1996; Hobbie *et al.* 2000). Warming temperatures have the potential to reverse these trends by creating more aerobic conditions by increasing the depth of the water table through thawing permafrost (Benninghoff 1952) and increasing nutrient availability, both of which could increase microbial activity and SOM decomposition rates, along with net primary productivity (NPP). Microbial activity, litter inputs (NPP), and nutrient availability are all components of soil aggregate formation (Six *et al*. 1999). An objective of this dissertation has been to test the applicability of the soil aggregate stability model of Six *et al*. (1999) to arctic soils.

Importance of physical stabilization of SOM and the risk of thawing permafrost

The importance of SOM stabilization within soil aggregates is well documented, particularly in grasslands and in agricultural systems of varying levels of cultivation intensity, from conventional tillage to no-tillage (Tisdall and Oades 1982; Elliott 1986, Six *et al*. 1998, 1999). Initially, tussock tundra may appear to share many characteristics with grassland soils, but they have many differences as well. Soils in both systems possess rich levels of SOM in mineral soils, relatively low levels of aboveground productivity when compared to forest systems, and wide-ranging seasonal climate conditions. On the other hand, the high SOM-containing mineral layer is overlain by a think organic soil horizon in tussock tundra. Much of this mineral layer exists as permafrost, thereby preventing access to frozen SOM. Permafrost prevents water movement into the soil profile, which creates an oxygen-poor environment, despite similar (or lower) levels of precipitation compared to grasslands.

Within permafrost soils, organic matter that is not part of the active pool may be decomposed if permafrost thaws to increasing depths (Zimov *et al.* 2006). Faster rates of microbially-mediated organic matter turnover combined with increased carbon availability may greatly increase atmospheric CO_2 concentration because particulate organic matter (POM) is generally not protected within aggregates in the permafrost layer. The decomposition of older SOM tends to be more temperature-sensitive than younger SOM (Kirschbaum 1995, 2006). Increasing temperatures could result in a loss of older, more stabilized carbon from arctic soils and temperature may also affect NPP, which could impact the rate of OM input into the soil.

Relationships between temperature and decomposition/NPP with regard to C storage. Soil organic matter stocks are inversely related to NPP at high latitudes, as there are large soil carbon pools in the Arctic, and a relatively low rate of NPP due to constraints on both production and decomposition. However, within the region, Low Arctic systems tend to be more productive and store more carbon than High Arctic systems, which are additionally constrained by low precipitation levels. Soil aggregate formation depends on an input of fresh organic matter (Six *et al*. 1999), so if those input rates are low, then older soil organic matter components may be used. In the case of the Arctic, a large pool of labile SOM that has undergone little decomposition exists in the soil. This fraction may form the basis for aggregate formation in arctic soils. However, increases in decomposition rates may result in a reduction of old, labile carbon in arctic soils. Carbon loss from warmed soils indicates that decomposition rates change faster than NPP rates (reviewed in von Lützow and Kögel-Knabner 2009). Increasing temperature warms the soil, which precipitates a chain of events that change ecosystem properties and carbon storage. Permafrost thaws, resulting in increased microbial and invertebrate mineralization rates of soil nitrogen and carbon at greater depths, increasing nutrient availability, the rooting zones of plants and the efflux of CO_2 (Grogan and Chapin 2000; Hobbie *et al.* 2002). Carbon stored in permafrost will be lost through increased rates of decomposition and root respiration, which is linked to a thickening of the mineral soil active layer (Lee *et al.* 2010).

In Arctic soils, warming may affect aggregate size distribution. If decomposition rates exceed the rate of net primary production (NPP), then in the immediate short term an increase in aggregate formation would be expected, but as fresh litter stocks declined, aggregate formation rates would decrease (Six et al. 1999), resulting in less SOM protection and a subsequent loss of carbon. If NPP increases alongside the rate of decomposition, then fresh litter inputs would increase, which could result in no difference in carbon content. However, the stability of that carbon may change; rather than being incorporated into aggregates, it may remain free as part of light fraction organic matter, which is a relatively young, labile SOM fraction.

Changes in the location of particulate organic matter (POM) from being occluded within aggregates to existing as free, non-aggregate-associated light fraction may affect arctic SOM stability. If aggregates do play a role in protection of SOM from decomposition, then light fraction organic matter would be the SOM fraction most vulnerable to decomposition while iPOM would be stabilized within aggregates. Additionally, if macroaggregate substructure provides increased levels of physical protection, then microaggregates within macroaggregates should contain higher levels of SOM than free microaggregates. As biological activity increases with depth in arctic soils (Gough *et al* in preparation), then the influence of biologically-mediated SOM stabilization mechanisms, such as aggregate formation will have applicability to increasingly larger pools of arctic soil carbon.

In the Arctic soils, changes in the light fraction will be a good indicator of SOM stability. If physical protection does play a role in stabilizing arctic SOM, once constraints on nutrient availability are removed, then light fraction organic matter could be decomposed. If, however, NPP rates increase with increasing nutrient availability, then increases in root growth may offset light fraction losses with nutrient addition. An increase in LF quality (lower C:N) would be indicative of increased LF decomposition because some LF carbon would have been consumed and respired whereas the nitrogen would have been conserved within the LF-associated microbial community.
Dynamics of arctic soil organic matter (SOM) storage may be influenced by changes in ecosystem structure and function (Oechel *et al*. 1993; Oechel and Vourlitis 1995; Clein *et al.* 2000. Determining the factors influencing soil carbon turnover in arctic soils is an important aspect in understanding carbon efflux between soil and atmosphere (Shaver *et al.* 2006). Warming may increase decomposition rates, making nitrogen more available, which would result in an increase in NPP and C storage (Hobbie *et al.* 1998; Shaver *et al.* 1992), which would constitute a negative feedback, thereby limiting carbon loss from the soil (Clein *et al.* 2000). Conversely, warming may increase the decomposition rate to

a greater degree than it does the NPP rate, resulting in a net loss of carbon (Oechel *et al.* 1993). The objective of my research was to examine potential mechanisms of SOM carbon loss/accumulation through physical stabilization in water-stable soil aggregates in the active mineral soil layer of Low Arctic tussock tundra in response to long-term nutrient addition.

The research described in this dissertation has focused on understanding how the aforementioned biotic and abiotic factors interact in the face of climate change to affect SOM dynamics in the soils of a changing Arctic. This goal was approached by studying the soils from native moist acidic tundra, with an array of plots from a long-term nutrient addition study. Plots included controls that received no nitrogen or phosphorus, and plots that had annual nutrient addition since 1989 and since 1996 with nitrogen and phosphorus (10 g N m^{-2} yr^{-1} as NH_4NO_3 and 5 g P m^{-2} yr^{-1} P as P_2O_5 each Spring) (Hobbie and Chapin, 1998). From soils collected from these plots over the 2006-2007 growing seasons, soil aggregate size distribution along with carbon and nitrogen content of aggregates and particulate organic matter fractions were measured. These variables among the treatments were compared, and studied in light of current conceptual models of aggregate/SOM dynamics (Six *et al*. 1999) with an eye towards understanding how predicted changes in climate might affect the distribution of organic matter in Arctic soils.

Section 1.4. 2006 Field Season Work

In order to determine the applicability of the Six *et al*. (1999) model to arctic soils, preliminary studies were developed to determine first, if biotically-mediated aggregate formation exists in arctic soils despite a short growing season, low temperature, and low oxygen availability due to intermittent water saturation, and second, the extent to which aggregates play a role on stabilizing soil organic matter. If water-stable aggregates exist in arctic soils and play a role in protecting SOM from decomposition, then soils with a high degree of structure would possess a higher carbon content than less-structured soils (Reviewed above). In 2006, soils from a long-term nutrient addition experiment (10 g N m^{-2} yr^{-1} as NH_4NO_3 and 5 g P m^{-2} yr^{-1} P as P_2O_5 each Spring) were collected from the Arctic Long Term Ecological Research (LTER) site at Toolik Lake, Alaska (Hobbie and Chapin 1998; Mack *et al*. 2004). There were two sets of nutrient addition plots – those fertilized since 1996 and 1989, as well as a control.

Soils from sample collections were separated into four water-stable aggregate size classes using the wet sieving method of Elliott (1986): large (>2000μm) and small (250-2000μm) macroaggregates, microaggregates (53-250μm), and a silt + clay fraction (<53μm). Soil aggregate mean weight-diameter (MWD) is a measure of average aggregate size and is an indicator of soil aggregation (van Bavel 1949). The MWD is the sum of the average size of each aggregate size fraction ($\bar{x}$) multiplied by the proportion (w) each fraction makes of the whole soil. The mean fraction diameters for large and small macroaggregates, microaggregates, and silt+clay were 5000, 1125, 151.5, and 26.5 μm, respectively.

$$MWD = \sum_{i=1}^{n} \overline{x_i}\, w_i \qquad \text{(Equation 1.1)}$$

At spring thaw, all soils had the same structural characteristics (MWD = ~850μm). Aggregates were present in all treatments; formation was not limited by the arctic climate. Aggregate MWD was greatest in control soils, but increased between the two sampling dates for all treatments ($p \leq 0.05$), which may indicate that aggregate turnover in the Arctic is a biologically-mediated process, dependent on fresh inputs of particulate organic matter (Fig 1.2). Both microbial activity and plant growth are stimulated by springtime nutrient flushes (Giblin *et al*. 1991; Wallensten *et al*. 2009), which may in part have driven aggregate formation. This mechanism of aggregate formation has been observed in other systems, including cultivated and grassland soils (Six *et al*. 2000).

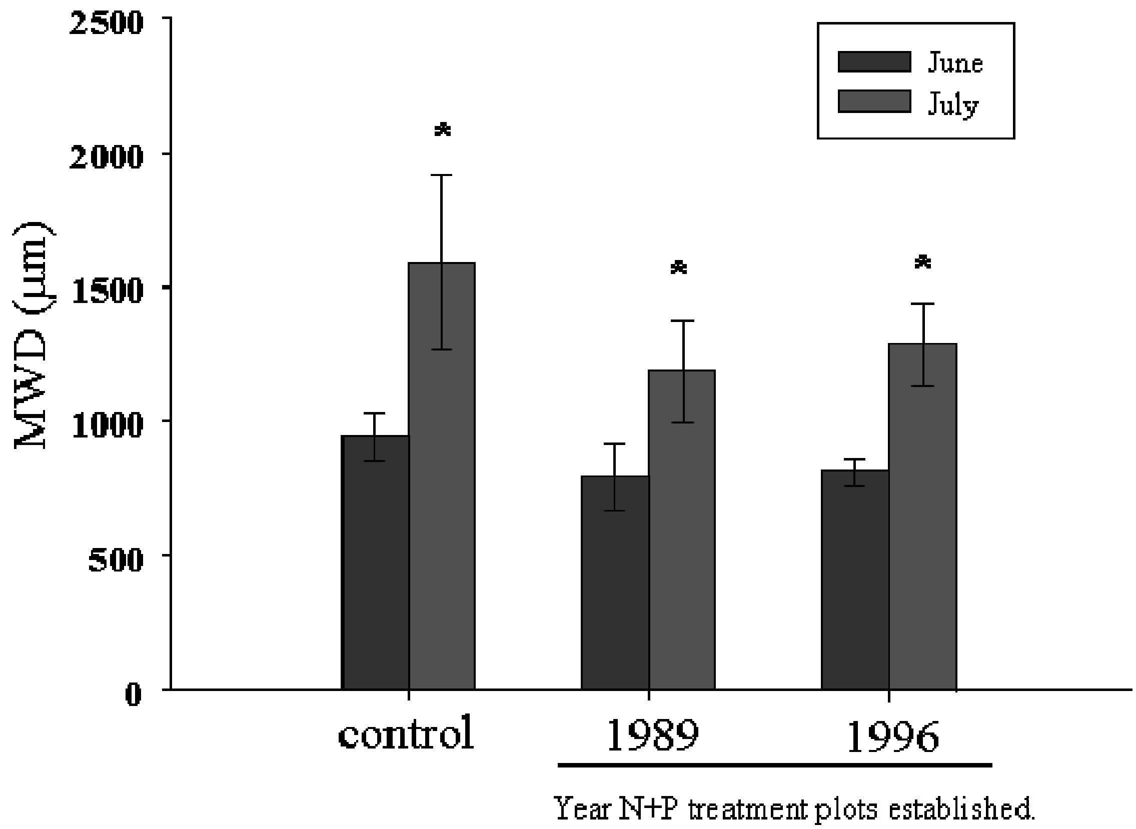

Figure 1.2. Aggregate mean weight-diameter (µm) of mineral soils sampled in 2006 from soils fertilized since 1989 and 1996, with control. Bars indicate mean ± standard error. Asterisks indicate significant date effect ($p \leq 0.05$).

The total organic carbon content of all soils and aggregate fractions collected in 2006 was measured. Carbon content increased over the growing season in control soils, but not in soils with nutrient addition. In addition, control soils measured in July had a higher carbon content than soils under nutrient addition since 1996 (Fig 1.3).

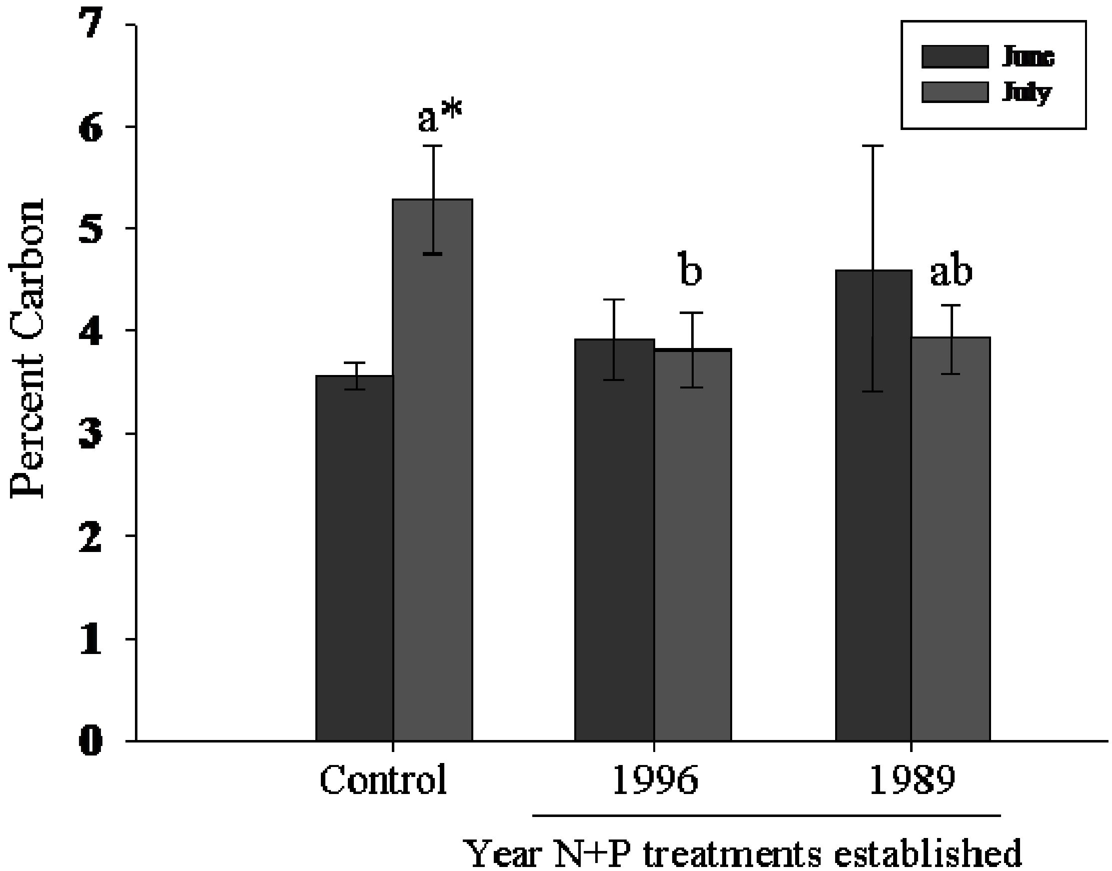

Figure 1.3. Soil carbon content (%C) of the top 10cm of mineral soils sampled in June and July 2006 from soils fertilized since 1989 and 1996, with control. Bars indicate mean ± standard error. Letters indicate treatment effect at $p \leq 0.10$. *Indicates date effect at $p \leq 0.05$.

These observations support the findings of Mack *et al.* (2004), who, using a July sample collection, demonstrated a net carbon loss in soils after 20 years of nutrient addition. The results from the June sampling used for this dissertation did not show a difference in soil carbon with nutrient addition. However, the reason for the large difference in soil carbon content, which is ten times the annual NPP (Shaver and Chapin 1991; Chapin *et* *al.* 1995) of moist acidic tundra, between control soils and soils with nutrient addition is unclear. This discrepancy may be due to sampling error, lateral movement of organic material, or possibly a downward movement of organic matter. It became clear that these soils may undergo shifts in carbon storage over the course of the growing season and that sample collections on multiple dates would be necessary in order to detect seasonal variation in soil aggregate and carbon storage dynamics.

Research conducted in 2006 demonstrated that water-stable aggregates exist in arctic soils, and that their formation is affected by nutrient addition, which also affects soil carbon content. Research in 2006 on soil carbon indicated a lower rate of carbon accumulation in soils with nutrient addition. It is possible that differences in aggregate formation dynamics may be linked to carbon storage in arctic soils.

Section 1.5. 2007 Field Season Work and Hypotheses

Results from the 2006 sampling suggested that linkages between aggregate structure and soil carbon storage may exist in arctic soils. The objective of the dissertation research for the 2007 field season was to separate arctic soils into several fractions, including aggregate-associated vs. unassociated (light fraction) organic matter and macroaggregate sub-structure elements (See Literature Review for a description), as well as the four aggregate classes that were separated in 2006 (see figure 1.1). In addition, plots were sampled taken multiple times in June 2007 in order to track temporal dynamics over the growing season, which lasts from early June through mid-August (Walker *et al*. 1999) Conceptually, the objective for the 2007 field season was to answer the following questions (From the Introductory section):

1. What linkages exist between soil C and macroaggregate sub-structure elements in the Arctic?
2. What are the temporal dynamics of aggregates in arctic soils over the growing season?
3. Is SOM more stable within aggregates than as non-associated light fraction?
4. What are the relationships between light fraction and live root biomass dynamics?

Soil fractionations and subsequent carbon and nitrogen measurements were separated into four hypotheses that were previously listed and are described in more detail here:

H1: *Aggregate size distribution in arctic tundra is dynamic under natural conditions, responding to changes in organic matter inputs, microbial activity, and temperature.*

Hypothesis 1 is predicated on established mechanisms identified in grassland and cultivated systems (e.g. Tisdall and Oades 1982; Elliott 1986; Six *et al*. 1999) along with preliminary research conducted at the Arctic LTER. The mechanisms at play include a positive relationship between aggregate size and carbon storage (Macroaggregates tend to store more carbon than microaggregates) as well as a link between microbial activity, substrate inputs, and aggregate formation. Increases in the rate of root growth and root exudate production due to nutrient addition would increase substrate availability. Increases in microbial activity would raise the rate of decomposition. The rates of both root growth and microbial activity are dependent on nutrient availability, and both would be enhanced by nutrient addition in a nitrogen-limited system. Low rates of decomposition lead to aggregate formation, while high rates result in aggregate disruption. This leads to the following predictions:

P1.1: Nutrient addition should alter aggregate size distribution dynamics. If decomposition rates are much greater than rates of organic matter input, then a loss of soil structure measured as a decline in macroaggregates and increase in microaggregates, resulting in a shift in aggregate size distribution could be observed over the growing season.

P1.2: If both input and decomposition rates increase as a result of nutrient addition, then soil structure may remain the same. However, if the input rate increases to a larger degree than the decomposition rate, the aggregate size distribution may shift resulting in an increase in macroaggregate formation over the growing season.

Hypothesis 1 was tested by collecting soil samples from a set of long-term nutrient addition plots at the Arctic LTER over the 2007 growing season. The soil was initially coarse-sieved (8mm) and air-dried, and later separated into four classes of water-stable aggregates.

H2: *Aggregate carbon and nitrogen content in arctic tundra are dynamic under natural conditions, responding to changes in organic matter inputs, microbial activity, aggregate size distribution, and temperature.*

Hypothesis 2 is based on positive linkages between aggregate formation/stability and SOM storage that have been established in other systems (e.g. Elliott 1986; Six *et al*. 1998). Macroaggregates tend to be more positively-associated with enhanced carbon storage than microaggregates; however, microaggregates are structurally more stable than macroaggregates. Therefore, disturbance tends to result in a loss of macroaggregates, and subsequently, a loss of soil carbon. Preliminary work conducted at the Arctic LTER provided evidence that reduced macroaggregate formation over the growing season was linked to a loss of soil carbon in soils under increased nutrient addition. Soils under the control treatment tended to contain more macroaggregates, which was due to increased macroaggregate formation and possibly to reduced rates of macroaggregate turnover (compared to soils with nutrient addition) and contained more carbon as well. These observations led to the following predictions:

P2.1: Nutrient addition should alter aggregate carbon and nitrogen storage dynamics by enhancing microbial activity, thereby increasing the rate of decomposition. As a result, aggregate turnover will be increased, resulting in increased SOM availability and carbon loss by the end of the growing season.

P2.2: If nutrient addition sufficiently enhances substrate production, then carbon content may not differ seasonally with nutrient addition because the rates of both incoming carbon (substrate production) and outgoing carbon (carbon dioxide efflux due to decomposition) may both be increased throughout the growing season, resulting in an equilibrium between input and output rates.

P2.3: If nitrogen is conserved within the soil, particularly in a nitrogen-limited system, then aggregate nitrogen content would most likely not change in response to increased inputs. However, if the rate of decomposition exceeds the rate of substrate input, then the amount of nitrogen relative to carbon may increase by the end of the growing season, resulting in a lower aggregate C:N.

Hypothesis 2 was tested by measuring the total carbon and nitrogen contents of each of the four water-stable aggregate size classes from soils that were collected from long-term nutrient addition plots at the Arctic LTER over the 2007 growing season.

H3: *Macroaggregate substructure size distribution and C:N in arctic tundra are dynamic under natural conditions, responding to changes in organic matter inputs, microbial activity, and soil macroaggregate turnover.*

In H3, the idea that linkages exist between SOM content and small macroaggregate substructure dynamics in low arctic systems (introduced above, in the 'aggregate sub-structure' section) is proposed. Microaggregate formation can occur within the structure of a macroaggregate (Oades, 1984; Golchin *et al*. 1994; Angers *et al.* 1997; Six *et al.* 1998). This occurs when microbes contained within a macroaggregate begin to decompose a piece of intra-aggregate particulate organic matter. Over time, oxygen becomes limiting, and the newly-formed microaggregate protects the iPOM within it (Oades 1984). This structure is further protected through its inclusion within the larger macroaggregate structure. Soils that undergo low rates of aggregate turnover tend to contain a higher amount of microaggregates contained within macroaggregates, which also contain higher carbon concentrations (Angers *et al*. 1997) than disturbed soils, where the microaggregates within macroaggregates become free microaggregates upon macroaggregate disruption. These concepts, based on previously established mechanisms (Angers *et al*. 1997; Six *et al.* 1999) led to the following predictions:

P3.1: Nutrient addition should alter macroaggregate substructure dynamics by stimulating the microbial community resulting in an increase in aggregate turnover over the growing season. Microaggregates occluded within macroaggregates in early June may enter the free microaggregate pool upon macroaggregate disruption later in the growing season.

P3.2: Nutrient addition affects the SOM content of microaggregates, which may decline as macroaggregate turnover increases as the growing season progresses.

Hypothesis 3 was tested by isolating three sub-macroaggregates (coarse POM, microaggregates within macroaggregates, and silt+clay contained within macroaggregates) from a subsample of previously-isolated small macroaggregates (described in H1 and H2) from soils that were collected from long-term nutrient addition plots at the Arctic LTER over the 2007 growing season. The proportion of each of these sub-fractions comprised of the macroaggregate was quantified, the carbon and nitrogen content of each sub-fraction was measured.

H4: *Particulate organic matter carbon content, nitrogen content, and location within the soil matrix as either light fraction or iPOM in arctic tundra are dynamic under natural conditions, responding to changes in organic matter inputs, microbial activity, soil aggregate turnover, and temperature.*

Organic matter inputs, including roots and root exudates are original sources of substrate for POM in soils. Light fraction organic matter is a POM fraction that has undergone some microbial processing, but is still a relatively young, labile fraction when compared to the total SOM pool (Molloy and Speir 1977; Molloy *et al*. 1977; Sollins *et al*. 1984; Theodorou 1990). Intra-aggregate particulate organic matter (iPOM) is contained within aggregates (Cambardella and Elliott 1992), but can re-enter the light fraction pool upon aggregate disruption. Free light fraction organic matter is not physically-protected within aggregates, and is more susceptible to decomposition, particularly with increased nutrient availability. These characteristics of light fraction and iPOM, in addition to those previously described in the 'light fraction organic matter' section above, led to the following predictions:

P4.1: Nutrient addition should have a lower impact on mineral-associated silt+clay carbon and nitrogen concentrations during the growing season than it would on POM fractions.

P4.2: Nutrient addition should alter light fraction carbon content dynamics. If new organic matter inputs increase due to active root decomposition, then the rate of material entering the light fraction pool will increase. At the same time, if the rate of light fraction decomposition increases with nutrient addition, then its contribution to the soil carbon pool may decrease by the end of the growing season.

P4.3: Nutrient addition should alter intra-aggregate particulate organic matter (iPOM) carbon content dynamics. If nutrient addition has resulted in shifts in the plant community, plant growth rates, and decomposition, then material being incorporated into aggregates (iPOM) could be reduced by the end of the growing season through a decrease in pool size (due to a loss of light fraction), or increased through increased aggregate formation as the growing season progresses.

P4.4: Nutrient addition should alter light fraction nitrogen storage dynamics. Increased root growth due to nutrient addition provides new material for microbial decomposition and subsequent incorporation into the SOM pool. At the same time, nutrient addition stimulates decomposition. Therefore, the proportion of aggregate and whole soil nitrogen that the light fraction comprises should increase over the growing season if the light fraction is being decomposed at a greater rate in soils with nutrient addition than control soils.

P4.5: If both the rate of light fraction formation and decomposition are increasing with nutrient addition, then over the course of the growing season, the light fraction C:N would decrease if decomposition exceeds formation, and would increase if formation exceeds decomposition. A lower C:N results from carbon being lost as carbon dioxide while nitrogen is conserved within the system.

P4.6: Root biomass dynamics are affected by nutrient addition. If root biomass increases, then there will be a seasonal increase in the amount of fresh, labile organic matter entering the SOM pool, comprising the light fraction and iPOM fractions.

Hypothesis 4 was tested by isolating the light fraction through density flotation from small macroaggregates and free microaggregates from soils that were collected from long-term nutrient addition plots at the Arctic LTER over the 2007 growing season, and quantified the carbon and nitrogen from these fractions. The heavy fraction was separated into mineral-associated carbon (silt+clay) and iPOM. The carbon content of the mineral-associated fraction was measured, and iPOM carbon content was determined through solving by difference. Because of the low nitrogen concentrations and instrument detection limits, it was not possible to determine iPOM nitrogen content.

The remainder of the dissertation is organized as follows: Chapter 2 of the dissertation focuses on H1, H2, and H3. Chapter 3 focuses on H4. In Chapter 4, potential linkages between the four hypotheses are discussed and a conceptual model of the relationships between them is proposed.

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CHAPTER TWO

FIELD SAMPLING, INITIAL AGGREGATE SEPARATION, AND MACROAGGREGATE SUB-FRACTIONATION

Section 2.1: Introduction

Soil organic matter (SOM) is formed through the decomposition of plant-derived material, dead consumers, and microbial by-products (Kögel-Knabner 2002). Controls inducing slow decomposition include low temperature and high levels of soil moisture (McKane *et al*. 1997; Hobbie *et al*. 2000). Over time, as decomposition progresses, labile materials are first consumed, followed by compounds of increasing recalcitrance. Decomposition may result in organic matter humification, which yields complex organic matter compounds that possess high chemical stability and long residence times in the soil (Kramer *et al*. 2003).

Arctic SOM is stabilized by low temperature and low oxygen availability (Hobbie *et al*. 2000), which is different from temperate SOM, which is stabilized through humification due to decomposition (Kramer *et al*. 2003). Soil organic matter is also stabilized though mineral associations (Feng and Simpson 2008), which occur in both arctic and temperate regions. Cold, anoxic conditions may limit decomposition in arctic soils, but rising temperatures may remove this constraint by increasing thaw depth, thereby lowering the water table, which persists on top of permafrost (Uhlířová *et al*. 2007). A large portion of the Arctic soil carbon pool consists of labile, relatively unmodified carbon that may be destabilized due to rising temperatures and increasing nutrient availability (Oechel *et al*. 1993), which would lead to increased microbial respiration (Sollins *et al*. 1996). Understanding factors that affect decomposition rates, including physical protection of SOM within water-stable soil aggregates (Chapter One) are important for improving our understanding of future effects of warming on arctic ecosystems.

Water-stable soil aggregates (See Chapter One for a review) occur in both arctic and temperate soils. Macroaggregate formation in temperate soils is positively linked with SOM accumulation (Jastrow 1996; Six *et al*. 1999; Six *et al*. 2000; Plante and McGill 2002) because they restrict SOM access by microbes and soil fauna (Sollins *et al*. 1996; Christensen 2001). Physical stabilization of SOM through occlusion within soil aggregates is well documented (e.g. Elliott 1986; Six *et al*. 1998). Soils that undergo less physical disturbance (i.e., grassland and no-tilled cultivated systems) tend to possess larger aggregates and more carbon than soils that are disturbed, particularly conventionally-tilled soils (Tisdall and Oades 1982; Elliott 1986; Six *et al*. 1998, 1999).

Macroaggregates (>250μm) can be separated into sub-fractions, including microaggregates within macroaggregates. Microaggregate-within-macroaggregate-stabilized SOM adds a level of physical stability additional to SOM occlusion within macroaggregates (Oades 1984; Angers *et al*. 1997; Denef *et al*. 2001). When the macroaggregate is disrupted, the microaggregate, which is more stable, tends to stay intact longer, thereby providing a physical means of carbon stabilization (Oades 1984;

Angers *et al*. 1997). Carbon existing as intra-aggregate particulate organic matter (iPOM) within the macroaggregate – but not the microaggregate within macroaggregate - would be susceptible to decomposition upon macroaggregate disruption whereas the iPOM would remain protected within the microaggregate within the macroaggregate (Denef *et al*. 2001). Six *et al*. (2000) have shown that microaggregates contained within macroaggregates contain a large proportion of aggregate carbon and are an important component of soil carbon storage.

The focus of the research for this dissertation has been on measuring the size distribution and temporal dynamics of water-stable soil aggregates as a means of physical stabilization of arctic SOM. Six *et al*. (1999) developed a conceptual model of soil aggregate temporal dynamics which showed that reduced physical disturbance resulted in greater aggregate stability over time. Tussock tundra soils are annually disturbed through cryoturbation, so instead of direct physical disturbance, effect of disturbance through nutrient addition has been studied. Long-term nutrient addition has resulted in vegetation shifts from tussock to shrub tundra and a loss of soil carbon (Hobbie and Chapin 1998; Mack *et al*. 2004). It is important to distinguish that the model of aggregate stability developed by Six *et al*. (1999) used cultivation as a primary disturbance. The objective of this dissertation has been to test the applicability of the conceptual model of Six *et al*. (1999) to low arctic systems, using vegetation shifts and nutrient addition as disturbances.

This chapter includes a field site description, sampling methods, and laboratory analysis for aggregates size distribution (H1 from Chapter 1 and carbon and nitrogen content (H2 from Chapter 1) as well as macroaggregate substructure size fraction distribution with carbon and nitrogen content (H3 from Chapter 1). Light and heavy density fraction separation measurements, including intra-aggregate particulate organic matter (iPOM) are covered (H4 from Chapter 1) in Chapter 3. The hypotheses listed in Chapter 1 to be covered in Chapter 2 are listed here, along with predictions:

H1: *Aggregate size distribution in arctic tundra is dynamic under natural conditions, responding to changes in organic matter inputs, microbial activity, and temperature.*

P1.1: Nutrient addition should alter aggregate size distribution dynamics. If decomposition rates are much greater than rates of organic matter input, then a loss of soil structure measured as a decline in macroaggregates and increase in microaggregates, resulting in a shift in aggregate size distribution could be observed over the growing season.

P1.2: If both input and decomposition rates increase as a result of nutrient addition, then soil structure may remain the same. However, if the input rate increases to a larger degree than the decomposition rate, the aggregate size distribution may shift resulting in an increase in macroaggregate formation over the growing season.

H2: *Aggregate carbon and nitrogen content in arctic tundra are dynamic under natural conditions, responding to changes in organic matter inputs, microbial activity, aggregate size distribution, and temperature.*

P2.1: Nutrient addition should alter aggregate carbon and nitrogen storage dynamics by enhancing microbial activity, thereby increasing the rate of decomposition. As a result, aggregate turnover will be increased, resulting in increased SOM availability and carbon loss by the end of the growing season.

P2.2: If nutrient addition sufficiently enhances substrate production, then carbon content may not differ seasonally with nutrient addition because the rates of both incoming carbon (substrate production) and outgoing carbon (carbon dioxide efflux due to decomposition) may both be increased throughout the growing season, resulting in an equilibrium between input and output rates

P2.3: If nitrogen is conserved within the soil, particularly in a nitrogen-limited system, then aggregate nitrogen content would most likely not change in response to increased inputs. However, if the rate of decomposition exceeds the rate of substrate input, then the amount of nitrogen relative to carbon may increase by the end of the growing season, resulting in a lower aggregate C:N.

H3: *Macroaggregate substructure size distribution and C:N in arctic tundra are dynamic under natural conditions, responding to changes in organic matter inputs, microbial activity, and soil macroaggregate turnover.*

P3.1: Nutrient addition should alter macroaggregate substructure dynamics by stimulating the microbial community resulting in an increase in aggregate turnover over the growing season. Microaggregates occluded within macroaggregates in early June may enter the free microaggregate pool upon macroaggregate disruption later in the growing season.

P3.2: Nutrient addition affects the SOM content of microaggregates, which may decline as macroaggregate turnover increases as the growing season progresses.

Section 2.2. Procedures

Section 2.2 Subsection 1:Study Site

Field research was conducted at the Arctic Long Term Ecological Research (LTER) at Toolik Lake (Fig. 2.1), in the northern foothills of the Brooks Range in Alaska (68˚38’ N, 149˚34’ W, elevation 760 m). The area is dominated by tussock tundra. Moist acidic tundra (MAT) has an organic layer >20 cm thick overlaying a mineral soil with imbedded permafrost. Vegetation consists of graminoids (mostly *Eriophorum vaginatum*), deciduous shrubs (mostly *Betula nana*), evergreens (mostly *Ledum palustre* and *Vaccinium vitis-idaea*), and mosses (mostly *Sphagnum spp*., *Hylocomium splendens*, and *Aulacomnium spp.*) (Chapin *et al*. 1995; McKane *et al*. 1997).

Experimental plots within the moist acidic tundra were arranged in a randomized complete block design with four blocks. Within each block, nutrient addition plots (5x20 m, 10g N m^{-2} yr^{-1} as NH_4NO_3 and 5g P m^{-2} yr^{-1} as P_2O_5 each Spring) have been maintained since 1989 and 1996 (Hobbie and Chapin, 1998).

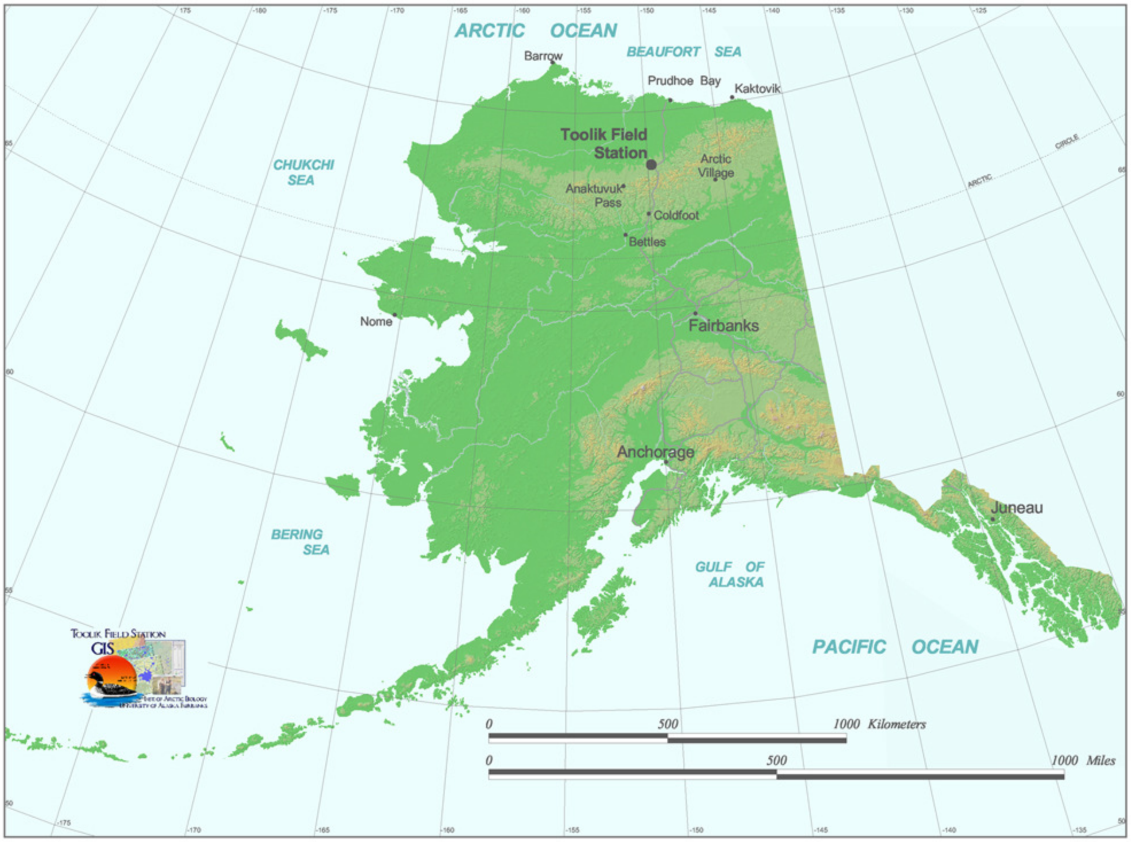

Figure 2.1. Location of Arctic LTER at Toolik Field Station, Alaska. Image created by Andrew Balser, University of Alaska, Fairbanks.

Section 2.2 Subsection 2: Site Description and Methods

1. Sample Collection

In 2007, soils were collected from a long-term nutrient addition experiment at the Arctic LTER at Toolik Lake, Alaska (Hobbie and Chapin 1998; Mack *et al.* 2004). Sample collections were made in early, mid, and late June, as well as August.

2. Laboratory methods

Soils were refrigerated at Toolik Field Station until they were sieved. Within a few days of sample collection, field-moist mineral soils were gently sieved through an 8 mm sieve.

Rocks, live roots, and dead organic matter were removed and quantified. The remaining soil was allowed to air dry. Soils were then transported to the Natural Resource Ecology Laboratory at Colorado State University for further fractionation and laboratory analysis.

Soils were separated into four water-stable aggregate size classes using the wet sieving method of Elliott (1986): large (>2000 μm) and small (250-2000 μm) macroaggregates, microaggregates (53-250 μm), and a silt + clay fraction (<53 μm). A 2000 μm sieve was placed in a pan filled with water so the mesh of the sieve was under ~1.5-2 cm of water. A 100 g soil sample was poured onto the mesh and sat undisturbed for five minutes to allow for slaking, which is the breakdown of unstable aggregates due to internal pressure changes upon submersion. The sieve was then moved up out of and back into the water 50 times in 2 minutes. The fraction remaining on top was poured into a pre-weighed pan and organic matter larger than 2000 μm was removed as this is not part of the soil organic matter pool (Six *et al*. 2002). The pan was oven-dried at 60˚C. Rocks larger than 2 mm were then removed. The remaining material constituted of the large macroaggregate (>2000 μm) fraction. Material that passed through the sieve was poured over a 250 μm sieve and the process repeated (except organic matter removal). The fraction remaining on the sieve constituted of the small macroaggregate fraction (250-2000 μm). Material that passed through the sieve was poured over a 53 μm sieve and the process repeated. The fraction remaining on the sieve constituted of the microaggregate fraction (53-250 μm). Material that passed through the 53 μm was centrifuged, the water decanted, and poured into a pre-weighed pan. This fraction constituted the silt+clay fraction. After oven-drying and weighing the fractions, sub-samples were finely ground for carbon and nitrogen analysis. Total organic carbon and nitrogen of all aggregate fractions were quantified by dry combustion using a Leco TruSpec CN analyzer (Leco Corporation, St. Joseph, Michigan).

Microaggregate Isolation:

Microaggregates contained within small macroaggregates (250-2000 μm) were isolated using the method of Six *et al*. (2000). A 10 g subsample was placed on top of a 250 μm sieve along with fifty glass beads (4 mm diam.). The sieve was gently shaken on a reciprocal shaker so that the macroaggregates were broken up with the aid of the beads. Continuous water flow through the sieve carried microaggregates through the sieve in order to not further disrupt them. Material passing through the 250 μm sieve was washed onto a 53 μm sieve, which was moved in water in the same manner as the initial aggregate separation procedure. This separation yields three fractions: the material remaining on the 250 μm sieve is considered to be coarse particulate organic matter (coarse POM); aggregates passing through the 250 μm sieve but retained on the 53 μm sieve are considered microaggregates isolated from within macroaggregates; and material passing through the 53 μm sieve is considered clay and silt particles not associated with stable microaggregates. All aggregate and POM fractions were dried (65˚C), weighed, and finely ground. Total organic carbon and nitrogen of all aggregate fractions were quantified by dry combustion using a Leco TruSpec CN analyzer (Leco Corporation, St. Joseph, Michigan).

Mean Weight-Diameter

The mean weight-diameter (MWD) presented in Equation 2.1 is a measure of average aggregate size (van Bavel 1949). The MWD is the sum of the average size of each aggregate size fraction $(\overline{x})$ multiplied by the proportion each fraction makes of the whole soil (*w*). The mean fraction diameters for large and small macroaggregates, microaggregates, and silt+clay were 5000, 1125, 151.5, and 26.5 µm, respectively.

$$MWD = \sum_{i=1}^{n} \overline{x_i} w_i \qquad \text{Equation 2.1}$$

<u>*Section 2.3 Subsection 3: Statistical methods*</u>

Field plots were arranged in a randomized complete block design (n=4) with nutrient addition and sample date as main effects. Control soils collected from block 1 in late June were not used in analysis because the samples collected at that time were not organic soils. All data were analyzed using SAS statistics software for analysis of variance (SAS Institute, 2003). Sub-samples were nested within treatment plots within each block. Block and block*treatment interactions were treated as random effects. Kenward-Roger degrees of freedom were used because the number of subsamples per plot varied from 1-3. Mean separations were tested using Tukey's honestly significant difference. Because of high natural heterogeneity in arctic soils, effects were considered to be significant at $p \leq 0.10$. Data were log transformed to meet assumptions of normality.

<u>Section 2.3. Results</u>

In the results section, data on aggregate size distribution and aggregate mean weigh-diameter (Hypothesis 1) are presented, followed by data on whole soil carbon, aggregate carbon and nitrogen, and carbon:nitrogen ratios for both whole soil and aggregates (Hypothesis 2). Then, similar data are presented for small macroaggregate-derived fractions, and end by comparing free microaggregates to those occluded within small macroaggregates (Hypothesis 3).

<u>Section 2.3 Subsection 1: Aggregate size distribution (H1)</u>

Aggregate size distribution did not differ with nutrient addition in early June, but did by mid-June when control soils had more macroaggregates and fewer free microaggregates than soils with nutrient addition. Control soils were comprised of significantly more large macroaggregates than soils with nutrient addition since 1989 and significantly more small macroaggregates than soils with nutrient addition since 1996. At the same time, there were fewer microaggregates in control soils than soils with nutrient addition since both 1996 and 1989. By late June and August, there were no longer differences in aggregate size distribution (Fig 2.2).

Control soils had a larger proportion of macroaggregates than soils with nutrient addition, which led to a greater mean weight-diameter than soils under long term nutrient addition (since 1989).

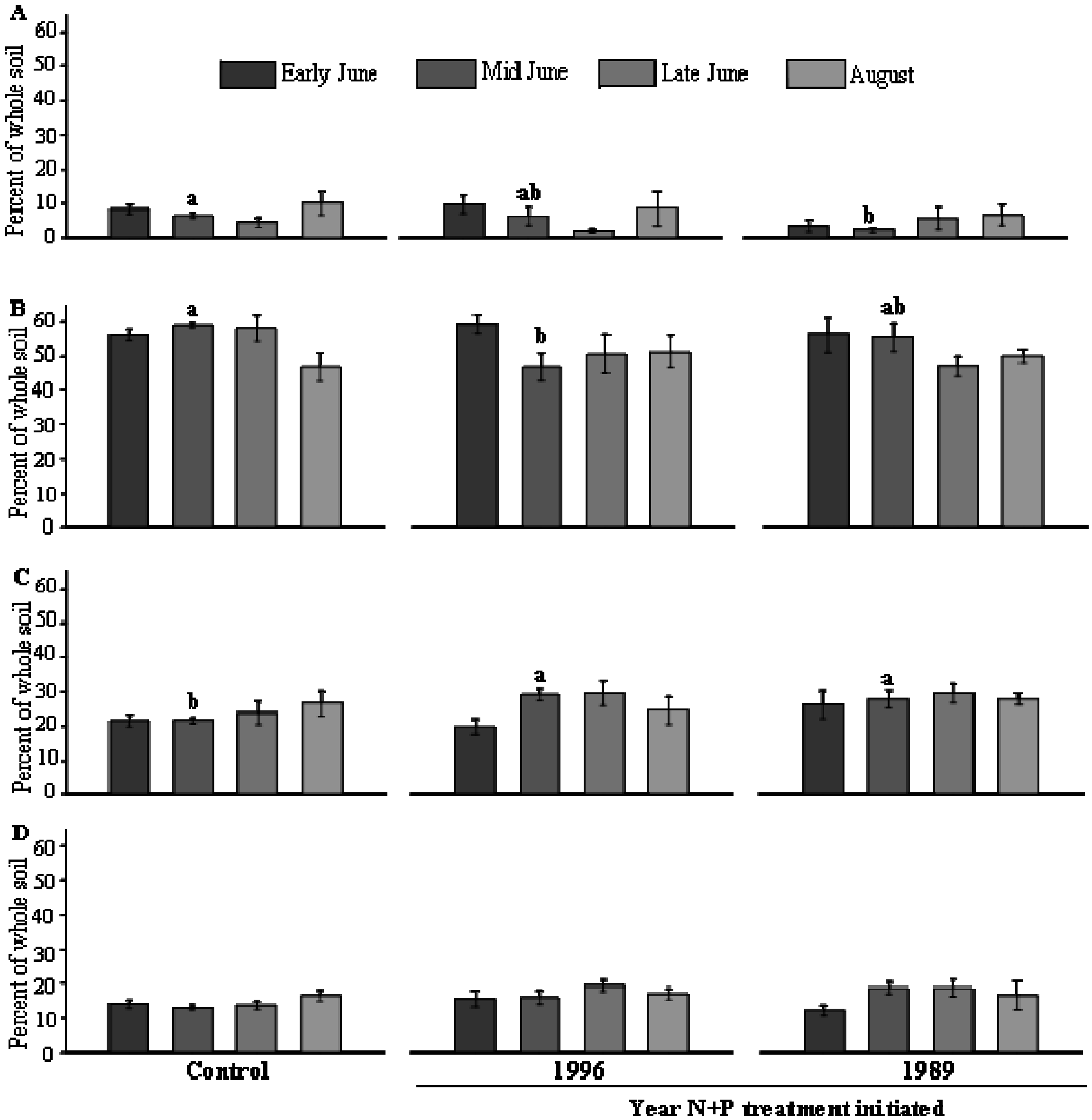

Figure 2.2 Water-stable aggregate size distribution. Uppercase letters indicate aggregate size fraction (A=Large Macroaggregates, B=Small Macroaggregates, C=Microaggregates, D=silt+clay). Bars represent mean ± standard error. Lowercase letters indicate significant differences between N+P treatments of the same size fraction ($p \leq 0.10$).

Control aggregate MWD was greater than soils with nutrient addition since 1989 (Fig. 2.3, $p \leq 0.05$). There were no significant date or treatment effects on MWD for soils with nutrient addition since 1996, but as a general pattern, the MWD decreased in these soils over the growing season. A similar pattern was measured in control soils, which differed from patterns observed the previous year (Chapter One). In soils with nutrient addition since 1989, the MWD remained constant throughout the growing season, which also differed from soils measured in 2006 (Chapter One). There were no significant effects of nutrient addition on the upper 15 cm whole mineral soil carbon content (Fig. 2.4). Furthermore, differences in carbon allocation and C:N ratios of soils were not observed. Whole soil carbon content did not differ significantly with nutrient addition (Fig. 2.4).

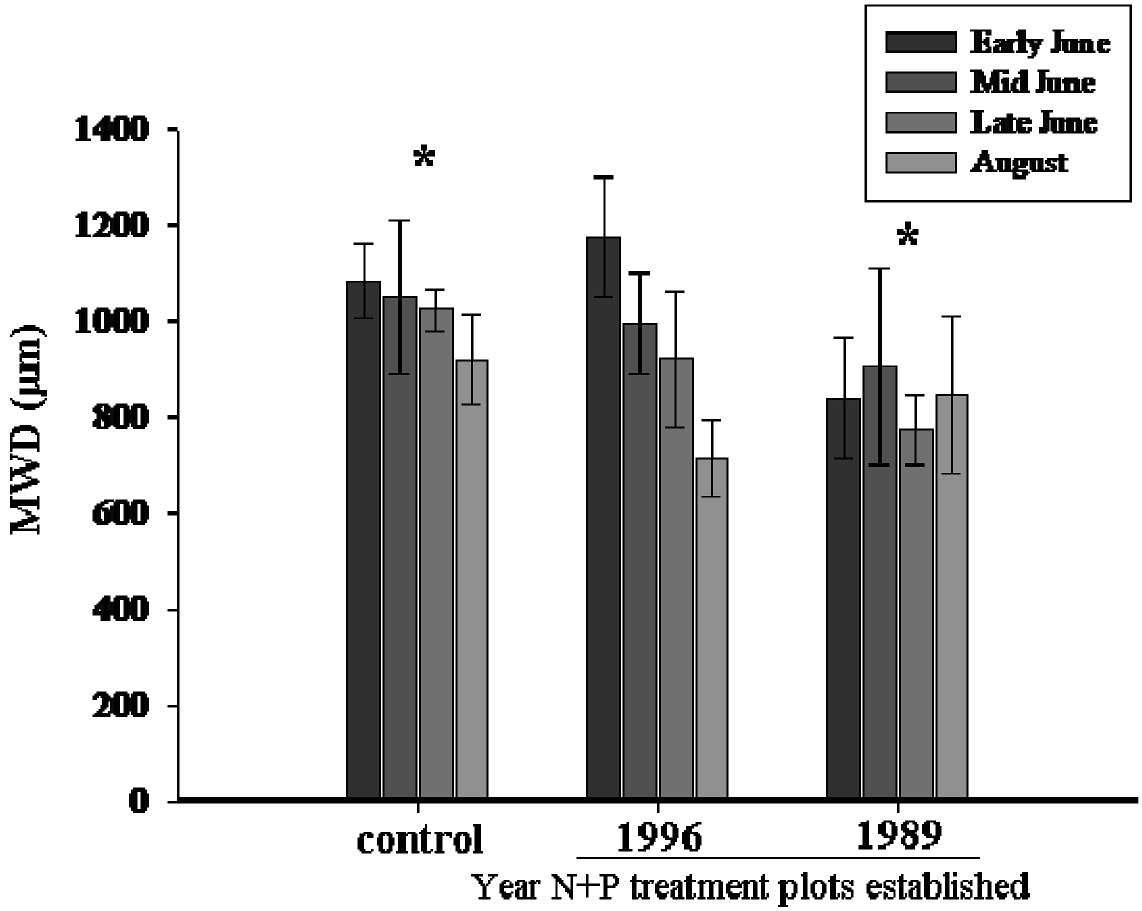

Figure 2.3. Mean weight-diameter (MWD) of water-stable aggregates collected from control and fertilized mineral soils (units = µm). Bars represent mean ± standard error. Asterisks indicate a significant seasonal average treatment effect ($p \leq 0.05$).

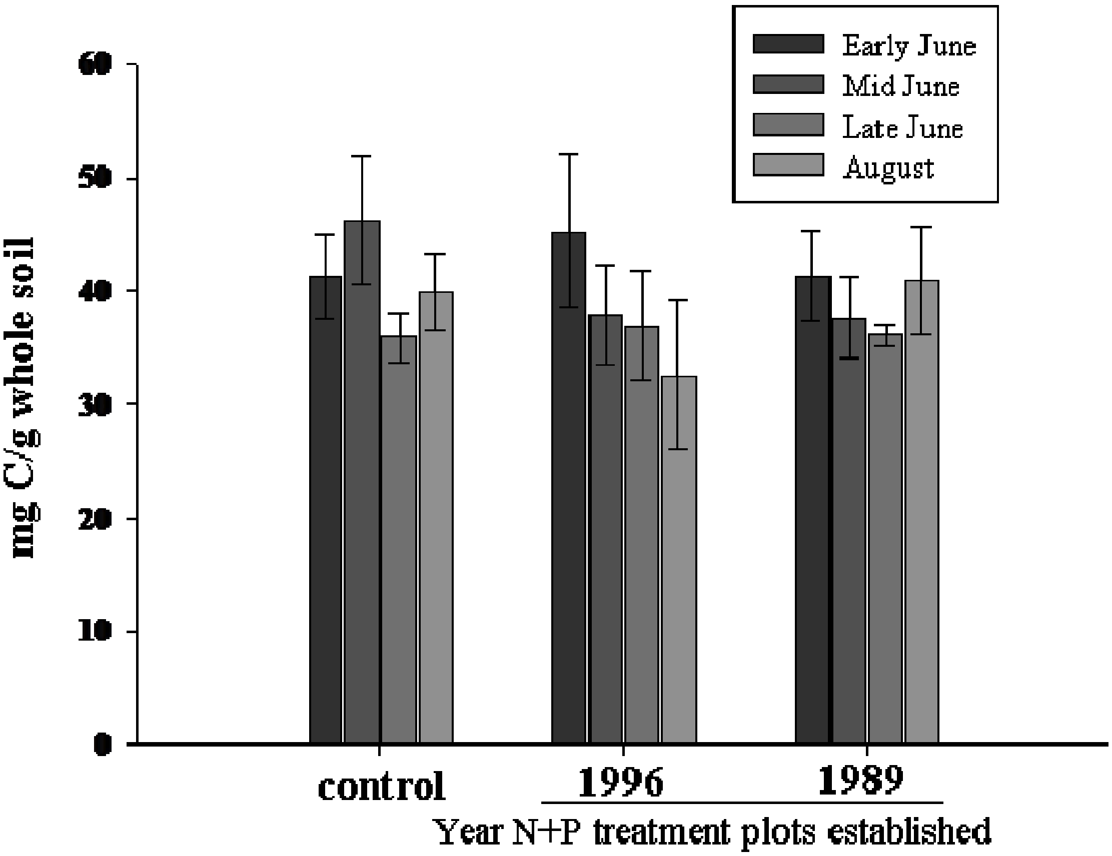

Figure 2.4. Whole mineral soil (15cm depth) carbon content (mg C/g whole soil) of control and fertilized mineral soils. Bars represent mean ± standard error. No significant effects.

Section 2.3 Subsection 2: Carbon and Nitrogen Results for aggregate size classes (H2)

The carbon concentration of large macroaggregates differed with nutrient addition. Large macroaggregates from soils with nutrient addition since 1989 had a significantly lower seasonal average carbon concentration than the control ($p \leq 0.05$) (Fig. 2.5). The large macroaggregate carbon content of soils with nutrient addition since 1996 did not differ from either the control or soils with nutrient addition since 1989. There were no treatment effects on carbon concentration in any of the other aggregate fractions.

There were more large macroaggregates in the control than in soils with nutrient addition since 1989, and they had a greater carbon concentration. This means on a whole soil basis, more carbon was associated with large macroaggregates in the control than in soils with nutrient addition since 1989 ($p \leq 0.05$) (Fig. 2.6). However, because this fraction made up a small proportion of the soil, procedural limitations prevented its separation into light fraction and heavy fraction components. This limitation on further large macroaggregate fractionation has been noted in other studies (Tan *et al*. 2007). The large macroaggregate fraction is less stable than small macroaggregates and is easily disrupted (Six *et al*. 1998). In all soils, the small macroaggregates comprised the largest aggregate fraction in term of abundance. This means on a whole soil basis, at least twice as much carbon was contained within the small macroaggregate fraction than in any other.

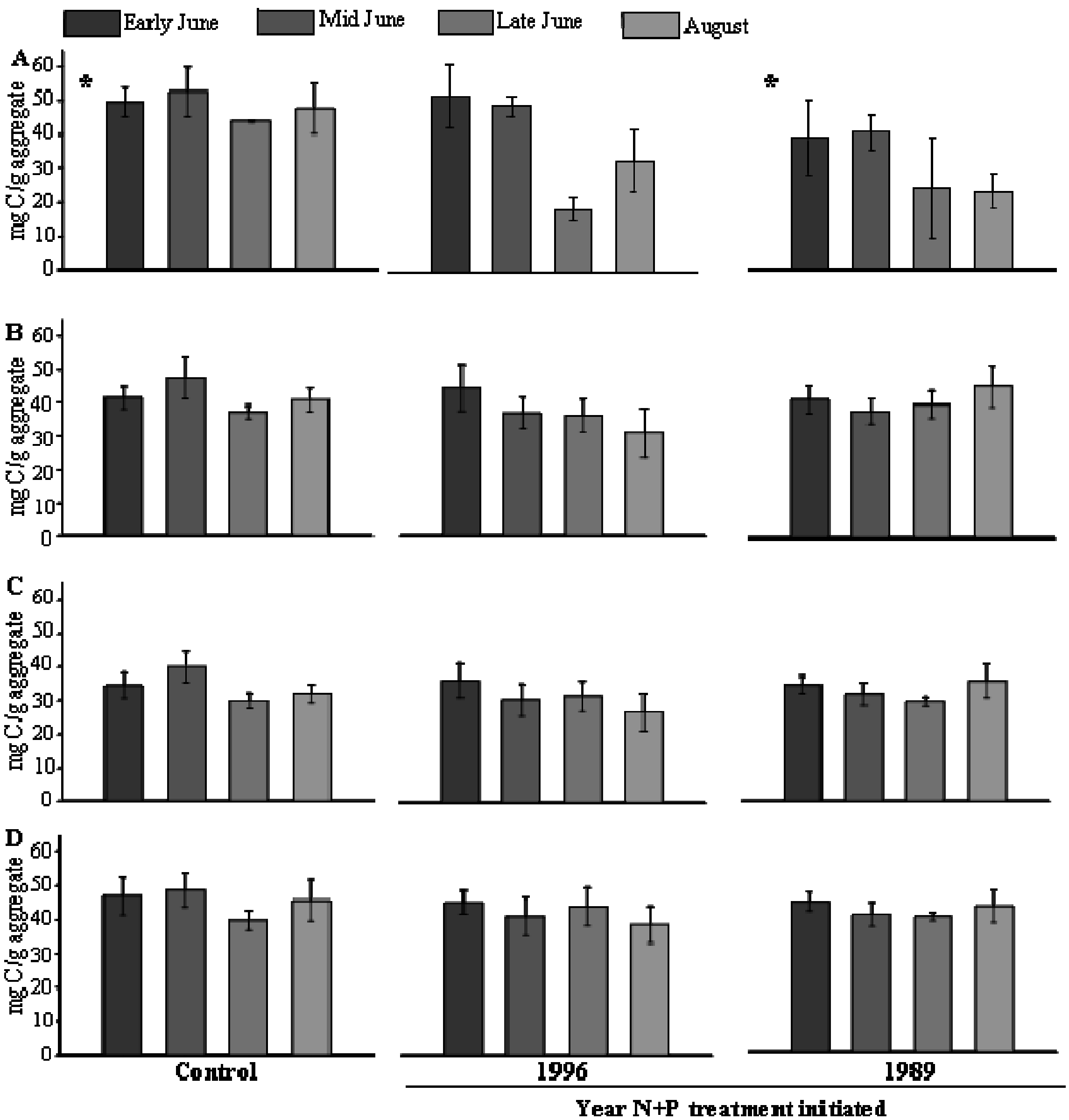

Figure 2.5. Carbon concentration of water-stable aggregates (mg C/g aggregate fraction). Uppercase letters indicate aggregate size fraction (A=Large Macroaggregates, B=Small Macro aggregates, C=Microaggregates, D=silt+clay). Bars represent mean ± standard error. Asterisks indicate significant differences between a seasonal average of N+P treatments of the same size fraction (p≤0.05).

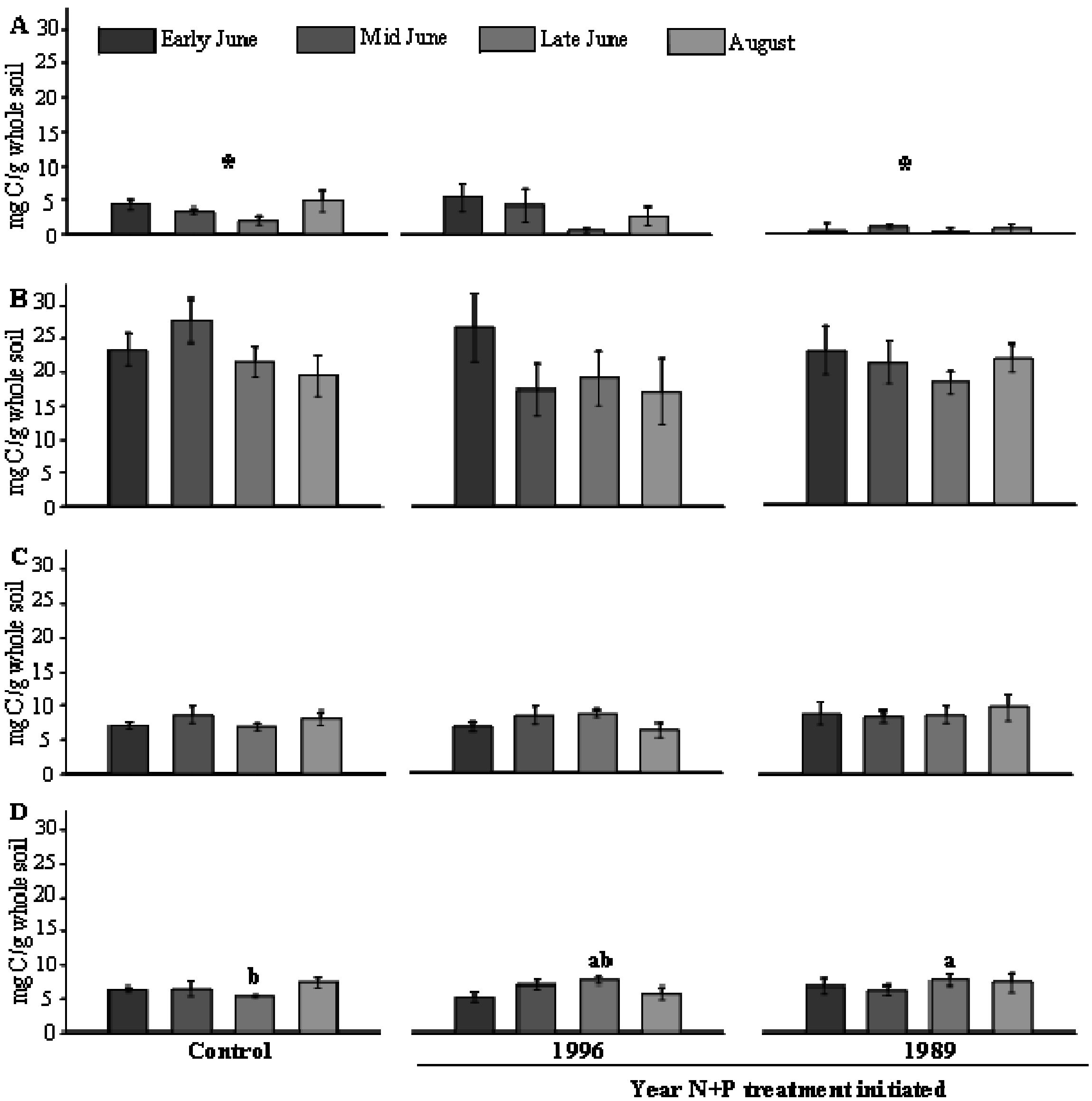

Figure 2.6. mg C/g whole soil in each aggregate fraction. Uppercase letters indicate aggregate size fraction (A=Large Macroaggregates, B=Small Macroaggregates, C=Microaggregates, D=silt+clay). Bars represent mean ± standard error. A seasonal average difference exists between the fractions marked with asterisks ($p \leq 0.05$). Lowercase letters indicate significant differences between N+P treatments of the same size fraction ($p \leq 0.10$).

Aggregate fraction nitrogen concentration differed only in large macroaggregates, where soils with nutrient addition since 1989 had, on a seasonal average, a lower nitrogen concentration than the control ($p \leq 0.10$, Fig. 2.7). The lower seasonal average nitrogen concentration in large macroaggregates from soils with nutrient addition since 1989 was due to a decline in nitrogen concentration over the growing season. The seasonal average nitrogen concentration of large macroaggregates from soils with nutrient addition since 1996 did not differ from the control or soils with nutrient addition since 1989. There were no treatment effects on nitrogen concentration on any of the other aggregate fractions.

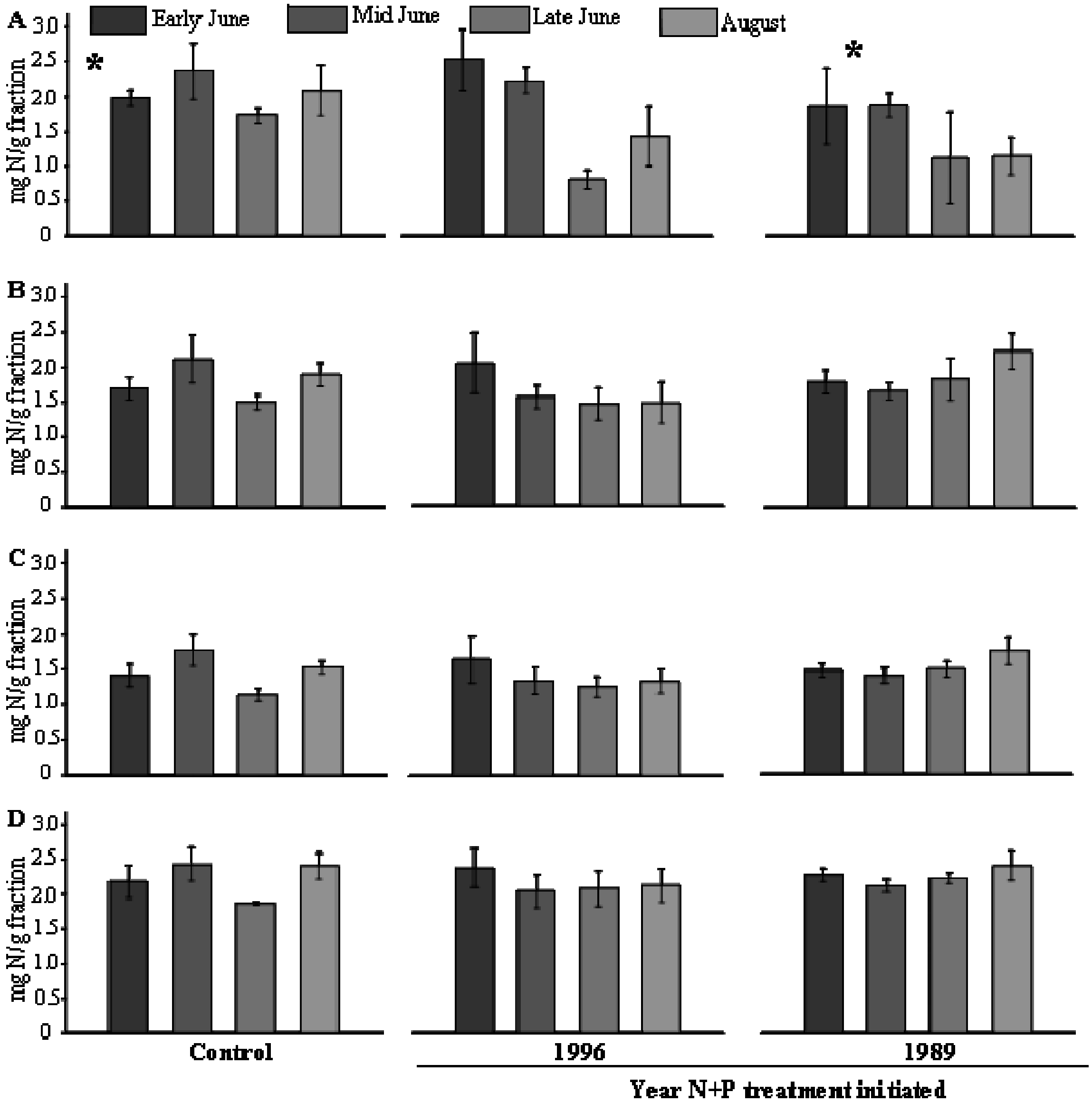

Figure 2.7. Nitrogen concentration of water-stable aggregates. Uppercase letters indicate aggregate size fraction (A=Large Macroaggregates, B=Small Macroaggregates, C=Microaggregates, D=silt+clay). Bars represent mean ± standard error. A seasonal average difference exists between the fractions marked with asterisks ($p \leq 0.10$).

Aggregate nitrogen content on a whole soil basis was affected by nutrient addition (Fig. 2.8). On a whole-soil basis, the large macroaggregate fraction contained more nitrogen in the control than soils with nutrient addition since 1989 as a seasonal average ($p \leq 0.05$)

and at the August sampling date ($p \leq 0.075$), but there were no treatment effects at any of the specific June sampling dates. Large macroaggregates from soils with nutrient addition since 1996 did not differ from the control or soils with nutrient addition since 1989 in terms of the amount of large macroaggregate nitrogen that was contributed to the whole soil nitrogen pool. However, the amount whole soil nitrogen content contributed by small macroaggregates was significantly affected by nutrient addition in mid June when the control soils contained more small macroaggregate nitrogen than soils with nutrient addition since 1996 ($p \leq 0.075$). Though there was a difference between the control and soils with nutrient addition since 1996, whole soil nitrogen content of small macroaggregates from soils with nutrient addition since 1989 did not differ from the control or soils with nutrient addition since 1996. Microaggregate whole soil nitrogen content was significantly affected by nutrient addition in late June when soils with nutrient addition since 1989 contained more small macroaggregate nitrogen than control soils ($p \leq 0.075$), but microaggregate whole soil nitrogen content of soils with nutrient addition since 1996 did not differ from the control or soils with nutrient addition since 1989. The whole soil nitrogen content of the silt and clay fraction was significantly affected by nutrient addition in late June when soils with nutrient addition since both 1989 and 1996 contained more nitrogen than control soils ($p \leq 0.075$). In all soils, the small macroaggregates comprised the largest aggregate fraction in term of abundance. This means on a whole soil basis, more nitrogen was contained within the small macroaggregate fraction than in any other.

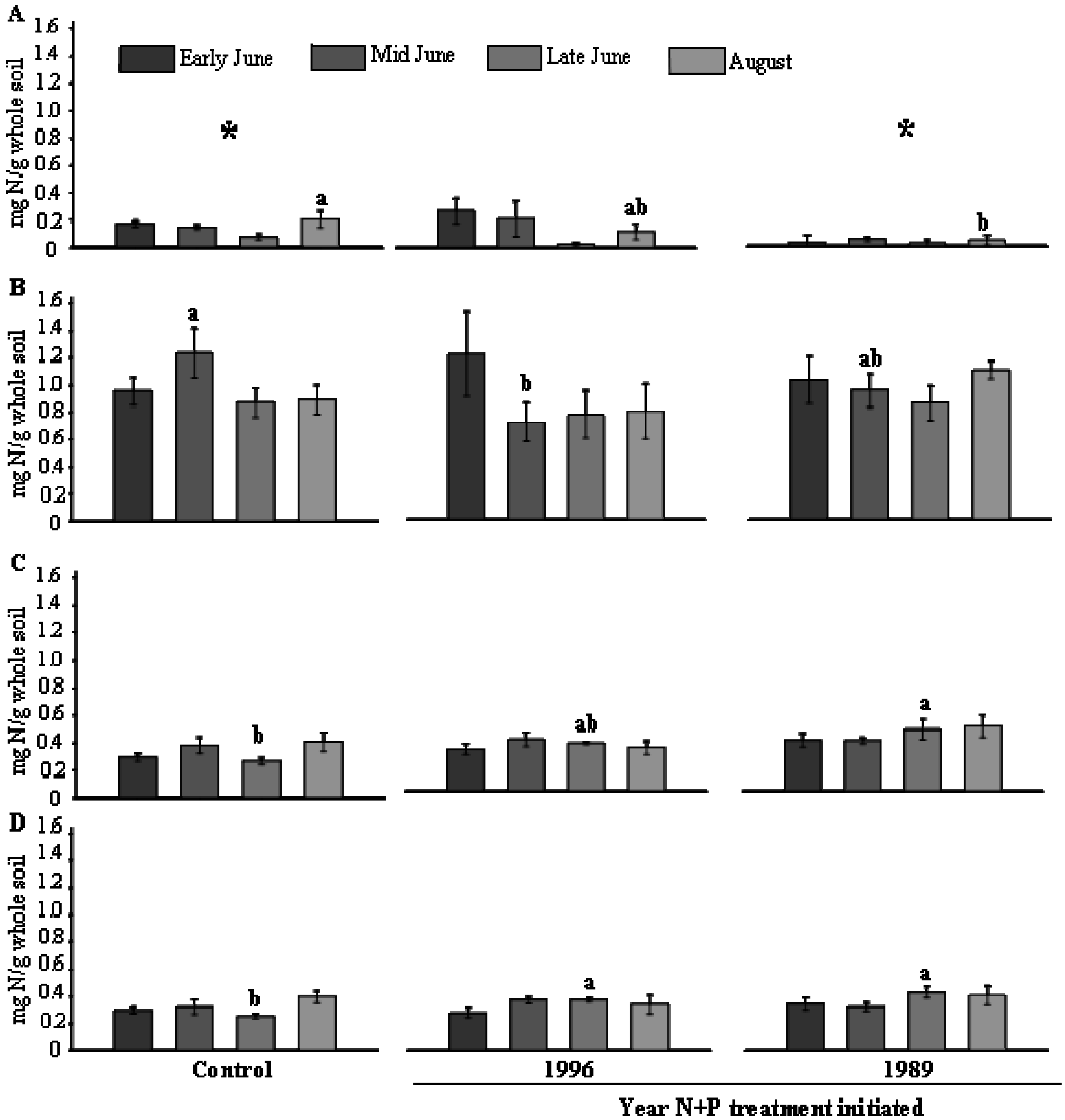

Figure 2.8. mg N/g whole soil in each aggregate fraction. Uppercase letters indicate aggregate size fraction (A=Large Macroaggregates, B=Small Macroaggregates, C=Microaggregates, D=silt+clay). Bars represent mean ± standard error. A seasonal average difference exists between fractions marked with asterisks ($p \leq 0.05$). Lowercase letters indicate significant differences between N+P treatments of the same size fraction and date ($p \leq 0.075$).

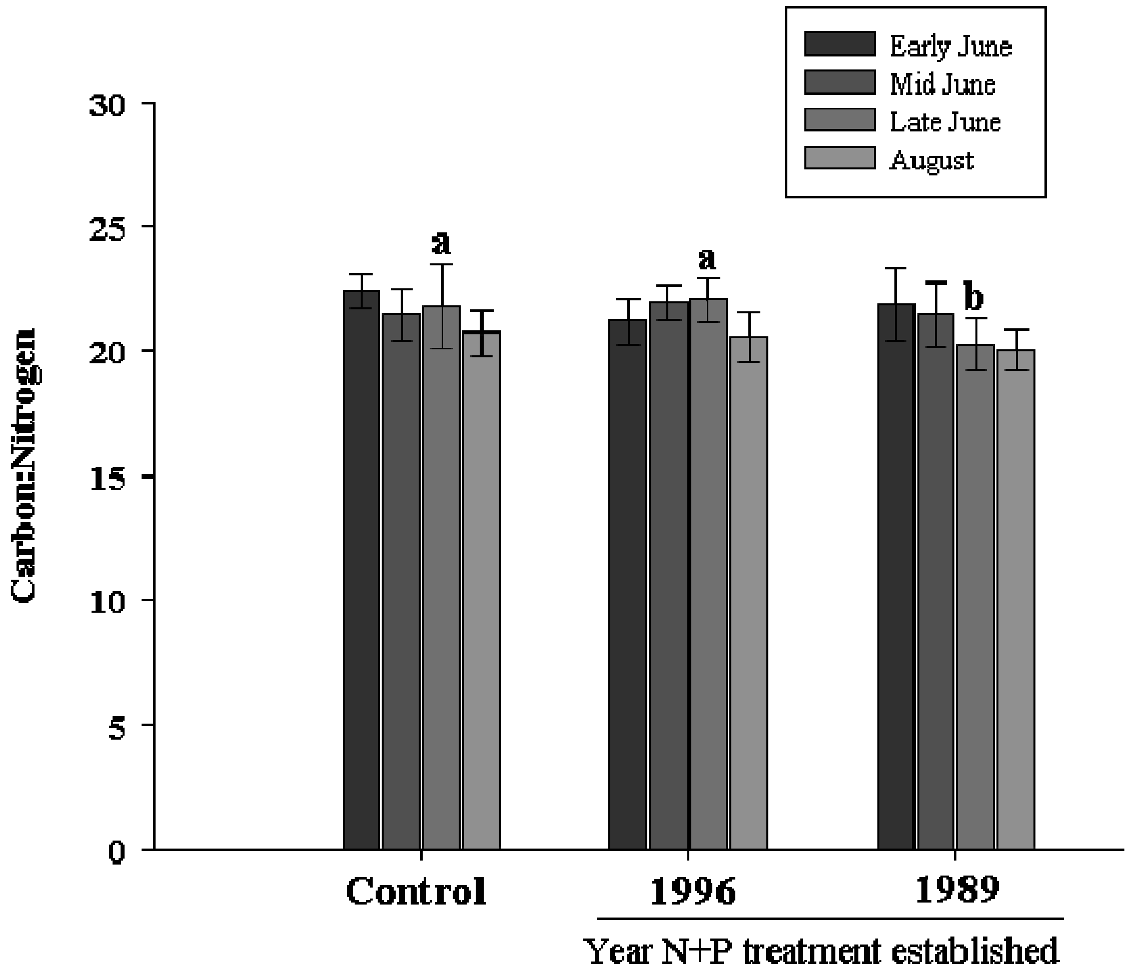

Figure 2.9. Whole soil carbon:nitrogen. Bars represent mean ± standard error. Letters indicate significant difference between fertilization treatments on the same sample date ($p \leq 0.075$).

Whole soil C:N declined over the growing season in all nutrient addition treatments, including the control ($p \leq 0.05$). In late June, whole soil C:N was also affected by nutrient addition, with soils with nutrient addition since 1989 having a lower C:N than soils with nutrient addition since 1996 and the control ($p \leq 0.075$, Fig. 2.9).

In general, the C:N of aggregates declined over the growing season ($p \leq 0.075$, Fig. 2.10), except for large macroaggregates from soils with nutrient addition since 1996. On a seasonal average, the large macroaggregate C:N was lower in soils with nutrient addition since 1989 than the control while the C:N of large macroaggregates from soils with nutrient addition since 1996 did not differ from the control or soils with nutrient addition since 1989 ($p \leq 0.10$, Fig. 2.10). However, due to low nitrogen levels and subsequent instrument (LECO) detection limits, only two blocks were used in late June for large macroaggregates from plots with nutrient addition since 1989. In late June, the C:N was lower in both microaggregates and silt+clay from soils with nutrient addition since 1989 than both the control and soils with nutrient addition since 1996 ($p \leq 0.05$, Fig. 2.10).

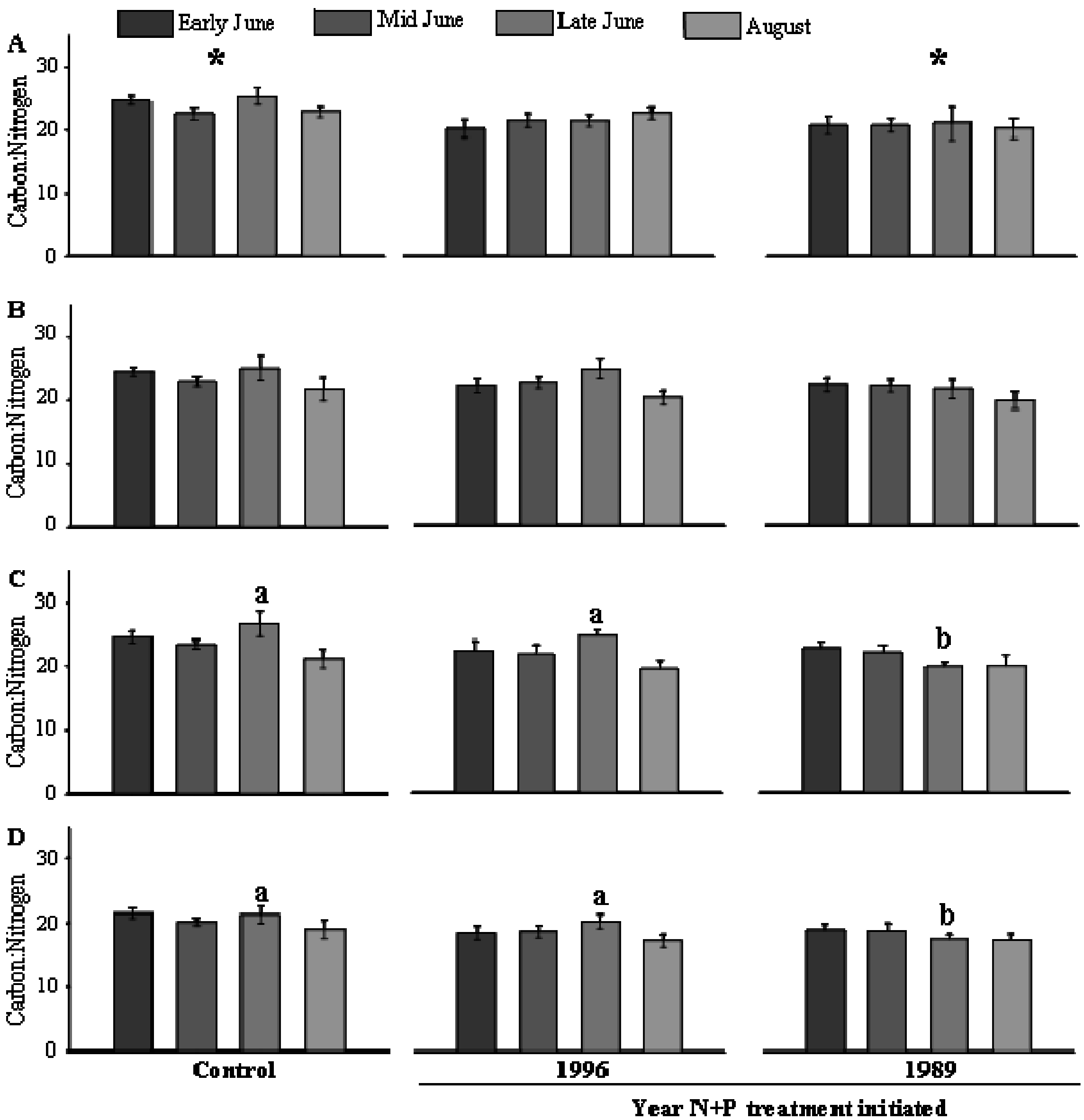

Figure 2.10. Carbon:Nitrogen ratio of water-stable aggregates. Uppercase letters indicate aggregate size fraction (A=Large Macroaggregates, B=Small Macroaggregates, C=Microaggregates, D=silt+clay). Bars represent mean ± standard error. Lowercase letters indicate significant differences between N+P treatments of the same size fraction ($p \leq 0.05$). A seasonal average difference exists between fractions marked with asterisks ($p \leq 0.10$).

<u>Section 2.3 Subsection 3: Small macroaggregate-derived sub-fractions (H3)</u>

In this section, the distribution of carbon and nitrogen content of macroaggregate-derived coarse sand and POM, microaggregates, and silt+clay fractions is described.

On a seasonal average, small macroaggregates in soils with nutrient addition since 1996 contained significantly more coarse sand and POM (>250 μm) and less silt+clay than the control ($p \leq 0.10$, Fig. 2.11). On a seasonal average, microaggregates comprised approximately 50% of macroaggregate mass, which is similar to southeastern US soils under no-tillage cultivation (Simpson *et al*. 2004) and soils of differing clay mineralogy (Denef *et al*. 2004) There was no difference in microaggregate-within-macroaggregate abundance with nutrient addition except in late June, where control soils contain significantly more macroaggregate-derived microaggregates than soils with nutrient addition since 1989 and 1996 ($p \leq 0.075$, Table 2.1). Macroaggregate-derived fractions did not significantly differ in carbon concentration with nutrient addition (Fig. 2.12).

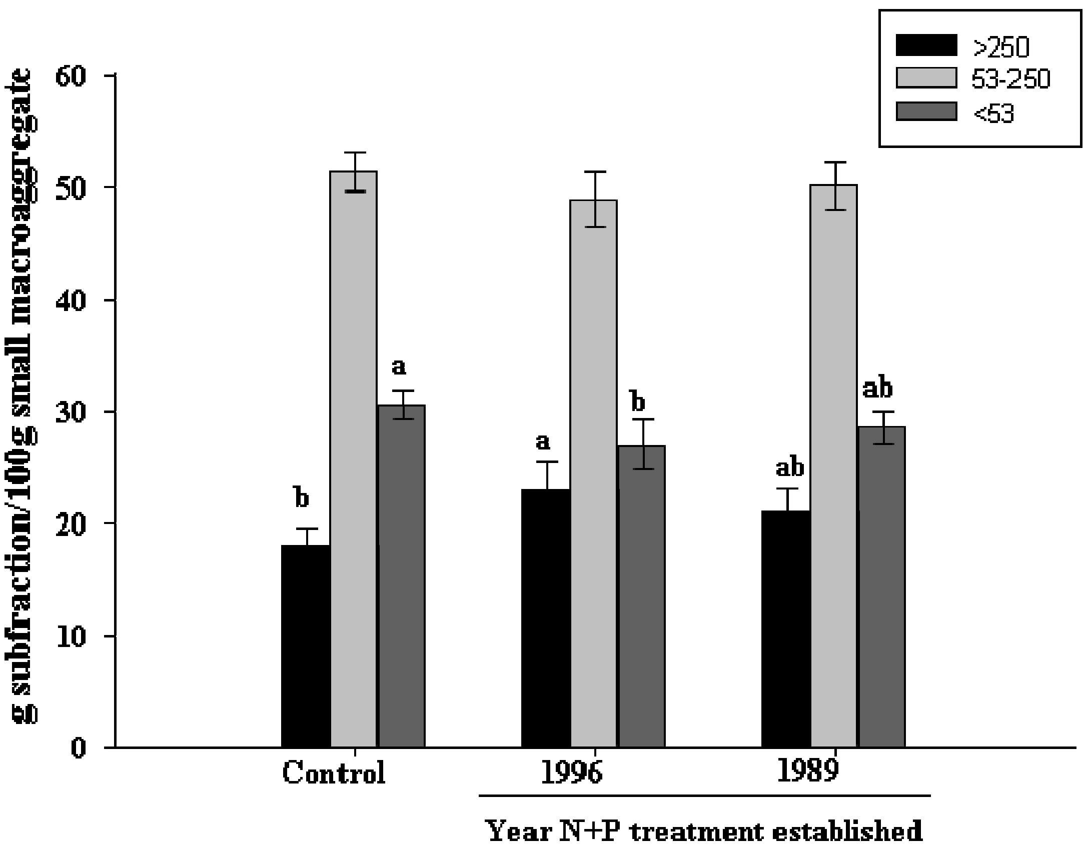

Figure 2.11. Seasonal average of the distribution of small macroaggregate sub-fractions: coarse sand and POM (>250µm), microaggregates (53-250µm), and silt+clay (<53µm) from arctic soils, mid-June 2007. Bars represent mean ± standard error. Letters indicate significant difference ($p \leq 0.10$) between treatments of the same aggregate sub fraction.

N+P treatment	% of small macroaggregate as microaggregates
Control	57.26 (1.09) a
Since 1996	49.71 (2.48) b
Since 1989	47.80 (0.93) b

Table 2.1. Percent of small macroaggregate (250-2000μm) weight as microaggregates (53-250μm) in Late June only. Standard errors are in parantheses. Letters indicate significant treatment effects (p≤0.075).

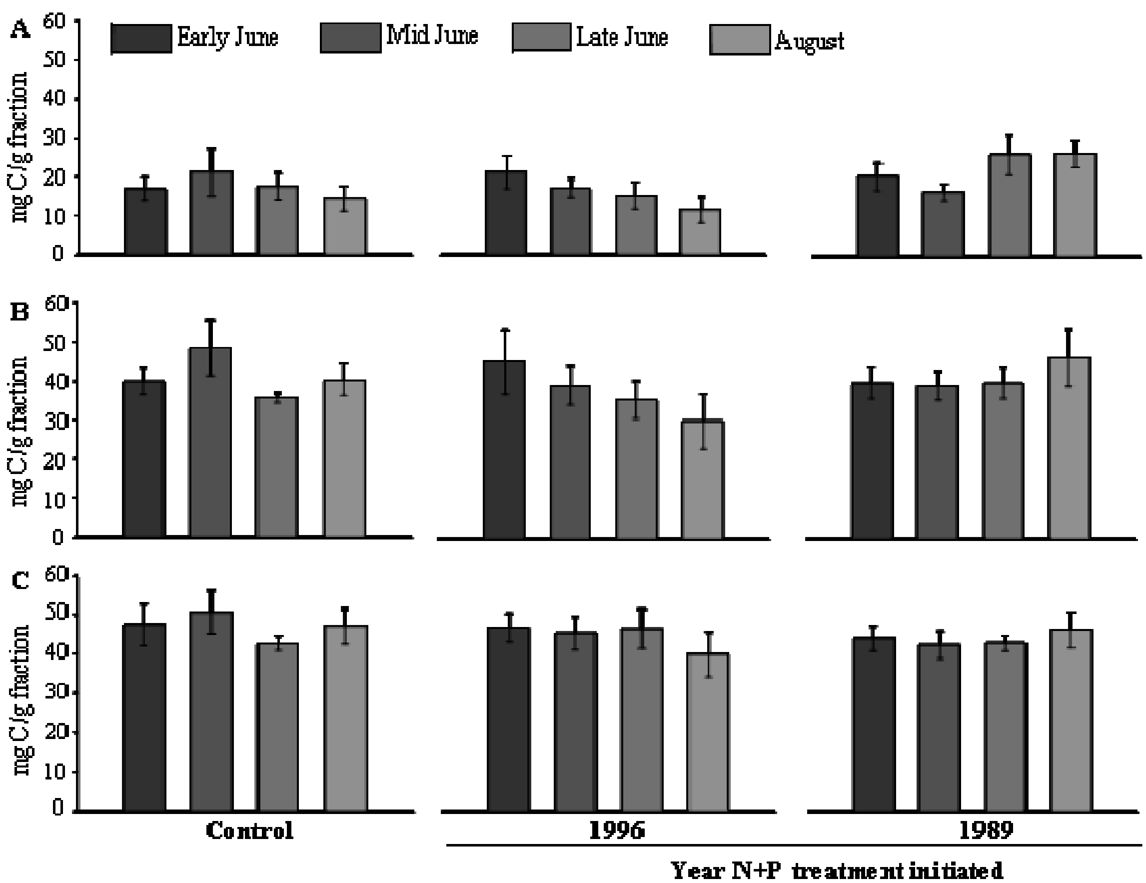

Figure 2.12. Carbon concentration in small macroaggregate subfractions (mg C/g fraction). Uppercase letters indicate aggregate size fraction (A=Coarse sand and POM, B=Microaggregates from small macros C=silt+clay). Bars represent mean ± standard error. No significant effects.

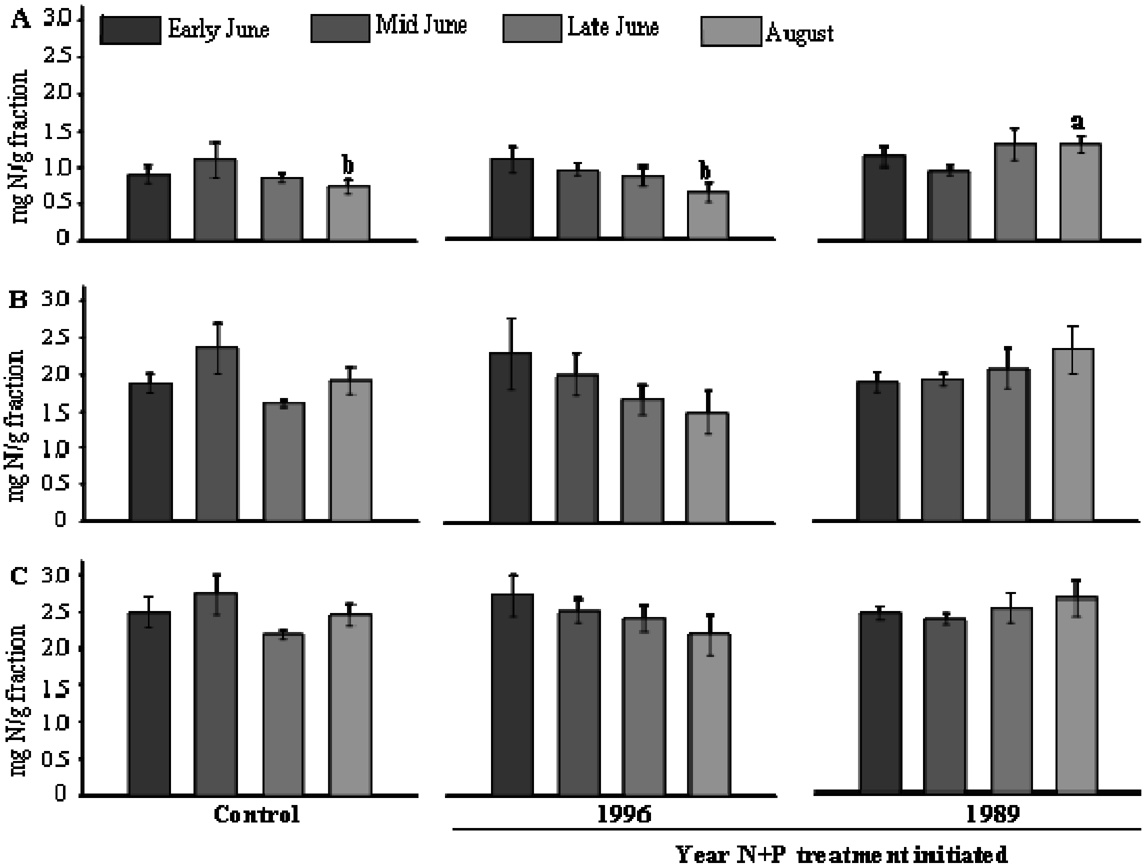

Figure 2.13. Nitrogen concentration in small macroaggregate sub-fractions (mgN/g fraction). Uppercase letters indicate aggregate sub fraction (A=Coarse sand and POM, B=Microaggregates from small macros C=silt+clay). Bars represent mean ± standard error. Lowercase letters indicate significant differences between N+P treatments of the same size fraction ($p \leq 0.05$).

Small macroaggregate sub-fraction nitrogen concentration did not differ significantly with treatment, except in August, when coarse sand and POM from soils with nutrient addition since 1989 contained a higher nitrogen concentration than the control and soils with nutrient addition since 1996 ($p \leq 0.05$, Fig. 2.13). Trends within the microaggregate-within-macroaggregate sub-fraction suggest that N concentration may have declined in soils with nutrient addition since 1996 while it increased in soils with nutrient addition

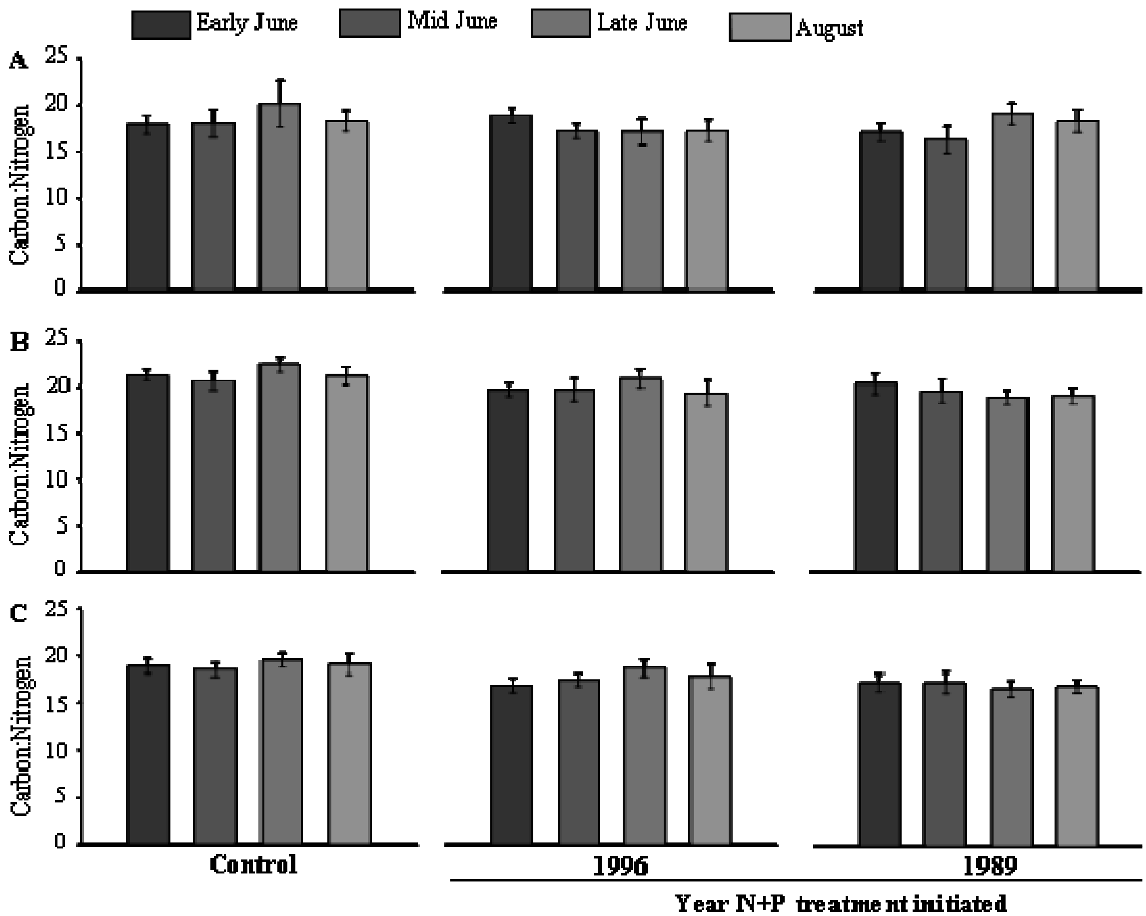

Figure 2.14. C:N ratio of small macroaggregate subfractions. Uppercase letters indicate aggregate size fraction (A=Coarse sand and POM, B=Microaggregates from small macros C=silt+clay). Bars represent mean ± standard error. No significant effects.

since 1989 over the growing season (Fig. 2.13). There were no significant differences in C:N in the macroaggregate sub-fractions with nutrient addition (Fig. 2.14).

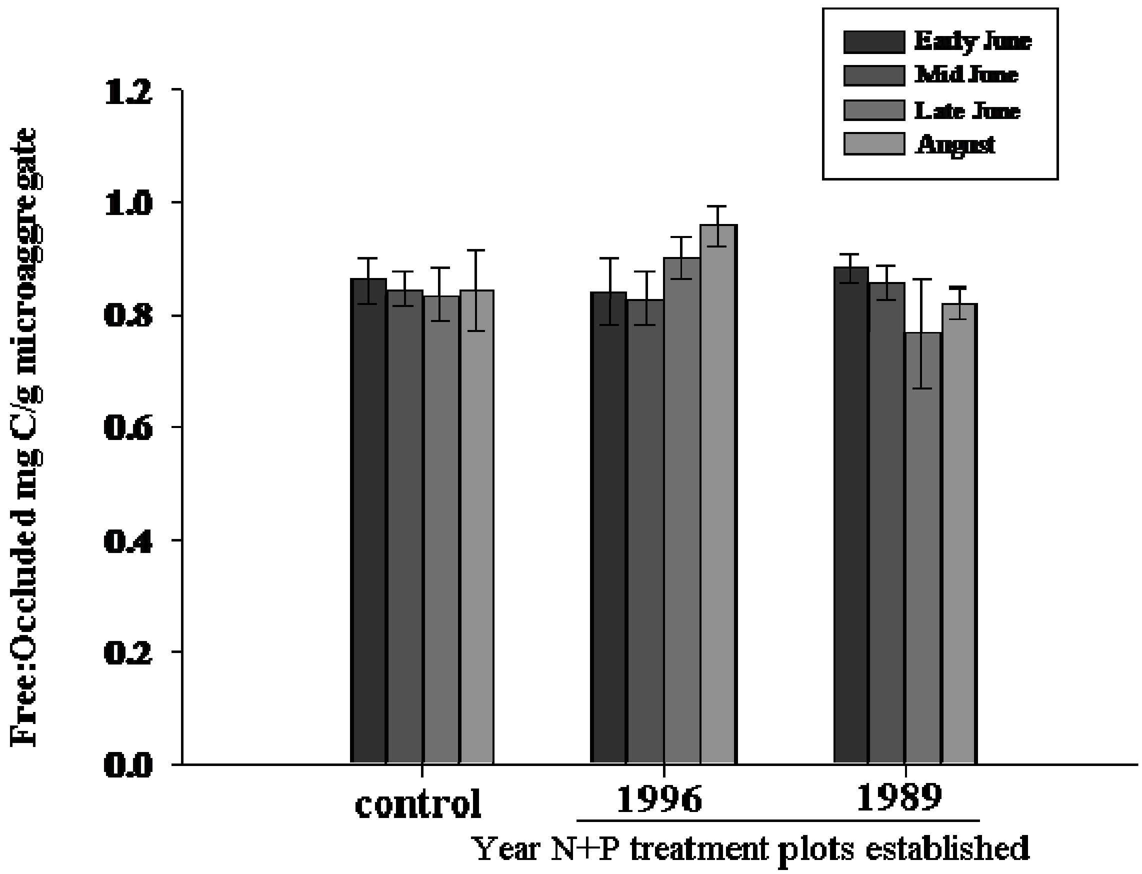

Figure 2.15. Ratio of free:occluded (within small macroaggregate) carbon concentration (mg C/g microaggregate). Bars represent mean ± standard error. No significant effects.

<u>*Section 2.3 Subsection 4: Comparisons between free and macroaggregate-derived microaggregates*</u>

In order to compare characteristics of free microaggregates with macroaggregate-derived microaggregates, the ratios of the abundance, carbon, nitrogen, and C:N ratios of free microaggregates over occluded microaggregates within macroaggregates were compared. If the ratio >1, then free microaggregates contained more C,N, or comprised more of the soil than macroaggregate-derived microaggregates. Occluded microaggregates had a

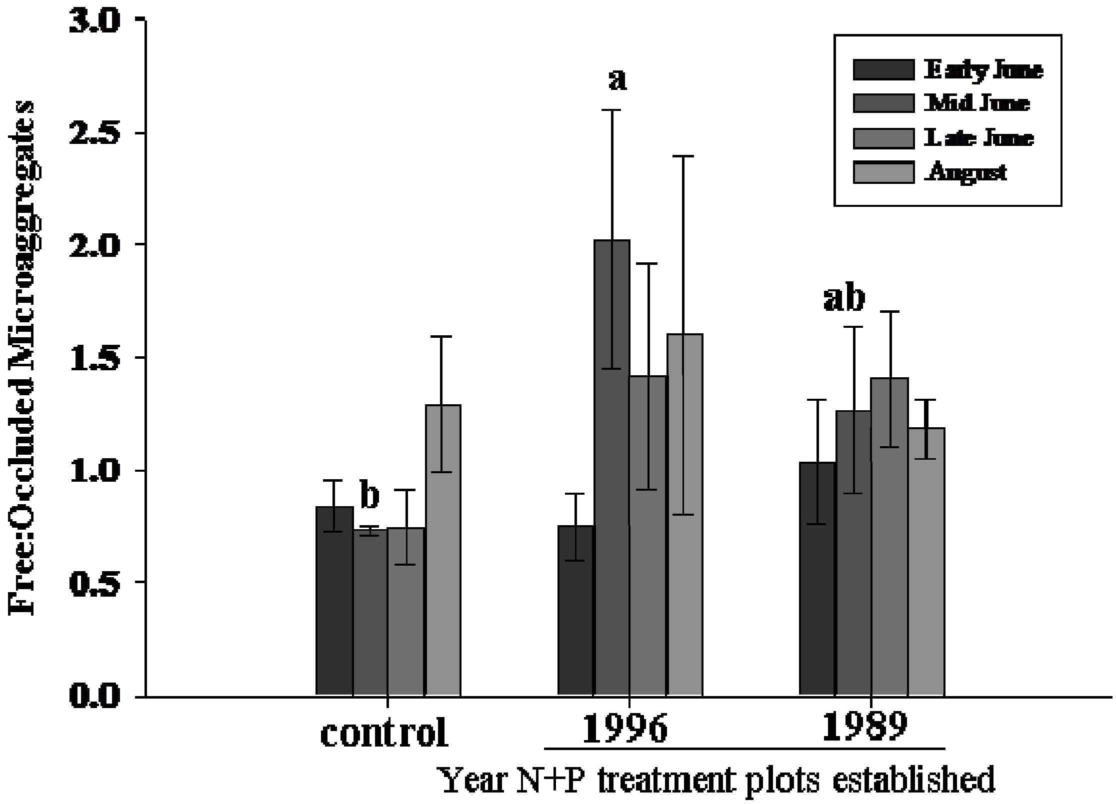

Figure 2.16. Ratio of Free:Occluded (within small macroaggregates) microaggregate abundance (g microaggregates/g whole soil). Bars represent mean ± standard error. Lowercase letters indicate significant differences between N+P treatments of the same sample date ($p \leq 0.075$).

greater carbon concentration than free microaggregates ($p \leq 0.075$), but there were no significant nutrient addition effects (Fig. 2.15).

Control soils tended to contain more occluded than free microaggregates in June, but by August, there were more free than occluded control microaggregates whereas the ratio of free:occluded microaggregates tended to increase in mid-June in soils with nutrient addition (Fig. 2.16). In mid-June, the free:occluded microaggregate ratio was significantly greater in soils with nutrient addition since 1996 than the control ($p \leq 0.075$).

The ratio of free:occluded microaggregate nitrogen concentration always remained under 1, meaning occluded microaggregates had a higher nitrogen concentration than free microaggregates ($p \leq 0.05$). However, the free:occluded nitrogen concentration ratio of soils collected in August was significantly ($p \leq 0.075$) greater than those collected in June (Fig. 2.17).

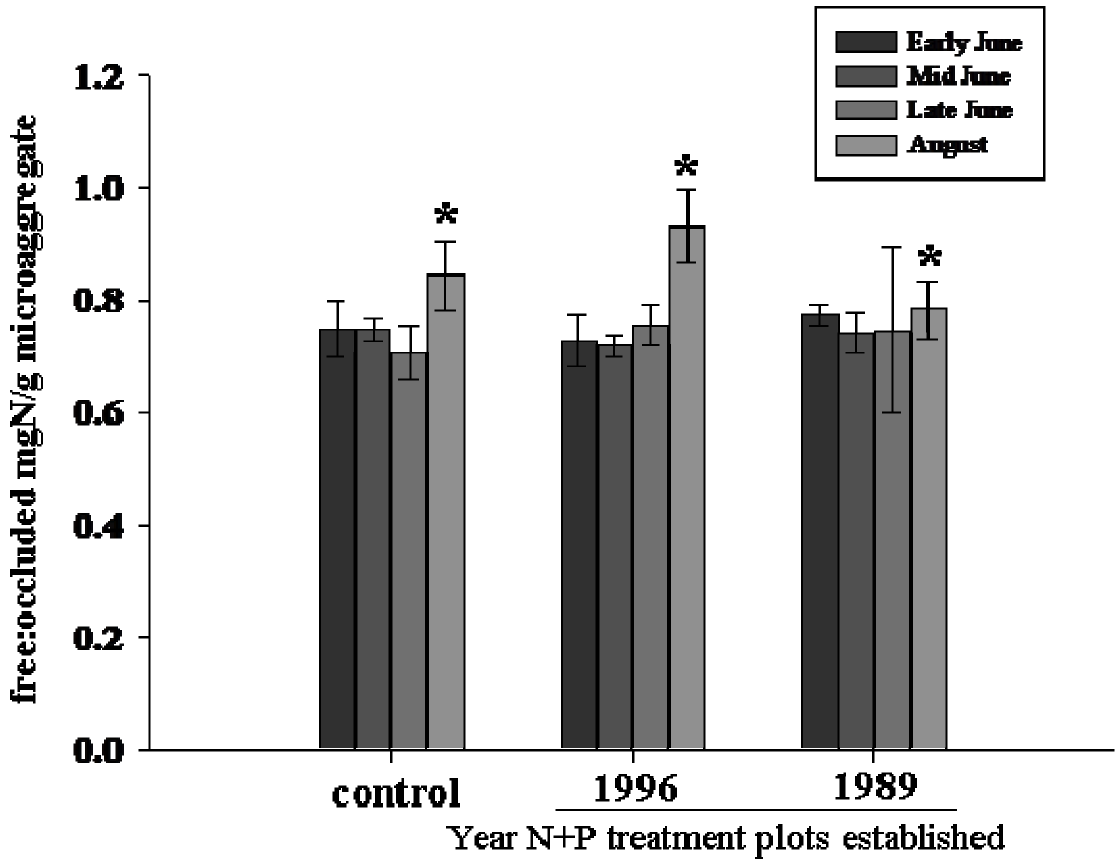

Figure 2.17. Ratio of free:occluded (within small macroaggregates) microaggregate nitrogen concentration (mgN/g microaggregate). Bars represent mean ± standard error. Asterisks indicate a significant date effect from other sampling dates for each fertilization treatment ($p \leq 0.075$).

Increases in free microaggregate nitrogen concentration combined with little difference in carbon concentration over the growing season resulted in a lowering of the free microaggregate carbon:nitrogen ratio relative to occluded microaggregates (Fig. 2.18) over the growing season. The free:occluded microaggregate C:N in August was lower than June sample dates for all nutrient addition treatments ($p \leq 0.05$).

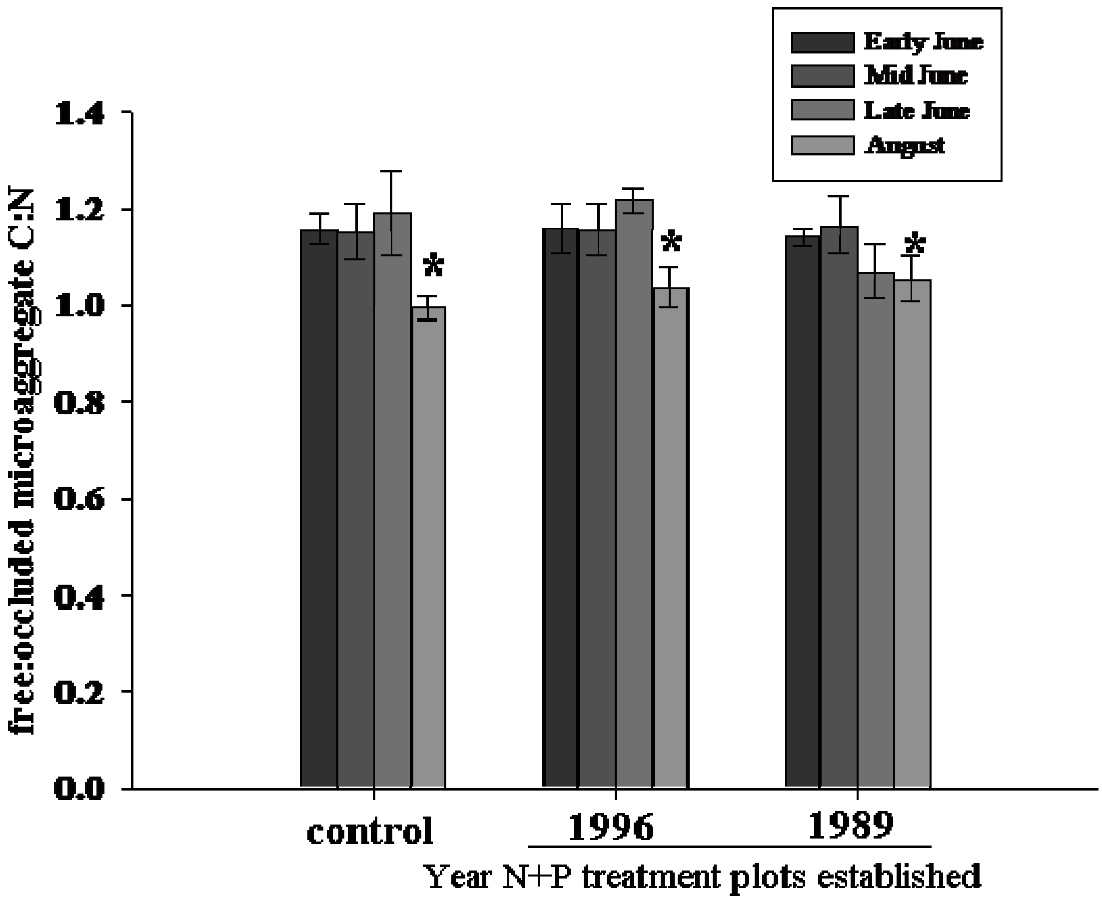

Figure 2.18. Ratio of free:occluded (within small macroaggregates) microaggregate nitrogen carbon:nitrogen. Bars represent mean ± standard error. Asterisks indicate a significant date effect from other sampling dates for each fertilization treatment ($p \leq 0.05$).

Section 2.4. Discussion

Section 2.4 Subsection 1: Aggregate size distribution (H1)

The objective of Hypothesis 1 was to determine if aggregate formation varies temporally over the growing season and with nutrient addition. Aggregate size distribution did not differ with nutrient addition at the beginning of the growing season. However mid-June aggregate distribution differed with nutrient addition. Control soils had more macroaggregates and fewer microaggregates than soils with nutrient addition. By the end of the season, however, there were no significant differences in size distribution with treatment. Mid-June soils were collected early in the growing season, approx. 15 days after spring thaw. These results suggest that the structure of Arctic soils is variable early in the growing season and undergo dynamic shifts in aggregation, but stabilize by the end of the season. On average, the distribution of aggregate size fractions was similar to those under cultivation in a temperate soil as measured by Six *et al*. (2002).

Though there were no treatment differences at the beginning or end of the growing season, nutrient addition effects during the growing season may result in periods of shifts in SOM stability. Long-term effects may result from these observed changes in soil structure. It is likely that both organic matter inputs and decomposition increased (Prediction 1.2), but these rate shifts have not had a definitive effect on soil structure aside from a seasonally average lower MWD in soils with nutrient addition since 1989 than the control (Fig. 2.3).

Section 2.4 Subsection 2: Aggregate C and N content (H2)

Nutrient addition (Hypothesis 2) did not affect whole mineral soil carbon or nitrogen content, which is contrary to the measurements of Mack *et al*. (2004), who measured a loss of carbon with long-term nutrient addition in both the organic and mineral layers from soils on a hillslope next to the one soils from the current study were collected. Prediction 2.1 described that aggregate carbon loss would occur in response to nutrient addition, and this was observed in large macroaggregates, but not in other fractions. Large macroaggregates in control soils had a higher carbon concentration and contributed more to the whole soil carbon than large macroaggregates in soils with nutrient addition since 1989 (Figs 2.5 and 2.6). However, larger macroaggregates comprised the smallest proportion of the total soil.

Microbial enzyme activity may be inhibited by nutrient addition if the amount of added nitrogen is sufficiently high. As a result, aggregate carbon content may be lower in these soils. Huang *et al*. (2010) found that manure applications increased aggregate carbon content. However, inorganic fertilizer applications did not. The present study was conducted on field plots that have received long-term inorganic fertilizer applications, and may have been affected similarly; at the same time, tussock tundra is nitrogen limited, so rather than having inhibitory effects on decomposition due to excess inorganic nitrogen, it is a lack of nitrogen that permits decomposition to take place (Wallenstein *et al*. 2009). The reason for a lack of change in aggregate carbon content in response to nutrient addition is unclear at this stage; however, later analyses on SOM allocation may provide information on how arctic SOM is impacted by nutrient addition (See Chapter 3).

Aggregate nitrogen concentration (Prediction 2.3) was not significantly affected by nutrient addition except in soils with nutrient addition since 1989, where the concentration was lower than the control. On a whole soil basis, there was more nitrogen allocated to small macroaggregates in control soils and more allocated to free microaggregates and silt+clay fractions in soils with nutrient addition since 1989 (Fig 2.8). This change in nitrogen allocation across aggregate size classes was due in part to significant shifts in aggregate size distribution in mid June, but effects observed in late June were due to a combination of shifts in nitrogen concentration and aggregate size distribution, neither of which were significant. However, when aggregate size distribution and nitrogen concentration trends were combined, significantly more nitrogen was allocated to smaller size fractions in soils with nutrient addition since 1989 than the control. Soils with nutrient addition since 1996 had a declining MWD, loss of carbon, and loss of nitrogen over the growing season, but none of these effects were significant. However, this may indicate that MWD, carbon, and nitrogen are related to each other, given that all three declined. In soils of all three treatments, higher MWD's tended to be associated with greater soil carbon content, which may indicate a positive association between these two factors, which has been described by others (e.g. Elliott 1986; Six *et al*. 1998). What is unclear is why the MWD declined in soils with nutrient addition since 1996, but did not in soils with nutrient addition since 1989. These results may indicate that responses to long-term nutrient addition may not proceed in a single direction, but exhibit dynamic qualities that may result in increases or decreases in soil structure over time.

Whole soil C:N was lower in August than previous sample collection dates across all nutrient addition treatments. In free microaggregates and silt+clay, soils with nutrient addition since 1989 had a lower C:N than the control. This lower C:N was due to an increase in nitrogen concentration and abundance of free microaggregate and silt+clay fractions, which also resulted in a lower MWD for soils with nutrient addition since 1989.

Whereas studies conducted in cultivated systems in temperate regions (e.g. Cambardella and Elliott 1992; Six *et al*. 1998, Six *et al*. 1999) have shown a consistent relationship between aggregate size, carbon/nitrogen content, and physical disturbance (disturbance reduces aggregate size and carbon content), nutrient addition in the Arctic does not seem to have the same consistent effect. However, Mack *et al*. (2004) reported a loss of carbon after 20 years of nutrient addition in arctic soils. A decline in carbon content was observed with nutrient addition in the preliminary work in 2006 (See Chapter 1), but no significant difference in whole soil carbon content in 2007 were measured, which may indicate that the response of arctic soils to nutrient addition in terms of aggregate distribution and carbon/nitrogen content is highly variable, and it is unlikely that the long-term carbon loss observed by Mack *et al*. (2004) was due to a linear decline in carbon content, but may have been due to large losses either initially, or during punctuated events during the growing season.

Section 2.4 Subsection 3: Macroaggregate-derived microaggregates (H3)

The distribution of microaggregates within macroaggregates in small macroaggregates (Prediction 3.1) did not differ except in late June, when control small macroaggregates contained a higher proportion of microaggregates within macroaggregates than both nutrient addition treatments. This result is similar to the findings of Simpson *et al*. (2004), who observed a decline in macroaggregate-derived microaggregate content in response to increased cultivation intensity in a temperate soil. In the present study, treatment effects observed earlier in the growing season were resolved with regard to soil structure. By the August sample date, neither aggregate size distribution nor occluded microaggregate content were affected by nutrient addition. Microbial activity tends to be greatest earlier in the growing season in these soils, when nitrogen availability is higher than later in the growing season (Giblin *et al*. 1991). If soil structure and microbial activity are related in arctic soils as they are in other systems (Beare *et al*. 1994, 1997; Bossuyt *et al*. 2001), then differences in aggregate distribution and formation would be expected when microbial activity is high.

On a seasonal average, soils with nutrient addition since 1996 contained more coarse sand and POM than the control, and carbon concentration distribution was not affected by nutrient addition. The carbon concentration (Prediction 3.2) in small macroaggregate sub-fractions was not significantly affected by nutrient addition, though occluded microaggregate carbon concentration declined (but not significantly) over the growing season in soils with nutrient addition since 1996 (Fig. 2.12). The nitrogen concentration in coarse POM of soils with nutrient addition since 1989 was significantly greater than other soils by the end of the growing season. Soils with nutrient addition since 1996 also had an increase in coarse POM nitrogen concentration, but this effect was not significant. This increase in nitrogen content in coarse POM with nutrient addition may indicate an increase in microbial activity, resulting in carbon loss and nitrogen conservation; however, there were no changes in the C:N of any of the macroaggregate sub-fractions.

Section 2.4 Subsection 4: Comparisons between free and occluded microaggregates

Comparisons were made between free microaggregates with microaggregates occluded within macroaggregates. Occluded microaggregates contained higher concentrations of both carbon and nitrogen than free microaggregates. Except for soils with nutrient addition collected in early June, soils with nutrient addition since both 1996 and 1989 contained more free than occluded microaggregates. Control soils were the opposite through the month of June, but by August, the ratio of free:occluded microaggregates was >1. At the same time, this ratio was also >1 for both nutrient additions, but was declining (Fig. 2.16). The C:N ratio was higher in free than occluded aggregates, but declined significantly by August in all treatments (Fig. 2.18). These results suggest that SOM quality (lower C:N) is greater in occluded than free macroaggregates, meaning that aggregate structure is important in stabilizing SOM in the Arctic. However, a lower C:N may be the result of increased SOM decomposition, which would result in a loss of carbon dioxide and nitrogen conservation, resulting in humification and an overall increase in recalcitrance. This increase in decomposition resistance may not be occurring at this point because even though the C:N is lower in occluded than free microaggregates, it is still >20:1, meaning nitrogen is still limiting in the system.

The results of small macroaggregate sub-structure fractionation suggest that the macroaggregate turnover rate for control macroaggregates increased by the end of the season, when their turnover rate was comparable to macroaggregates from soils with nutrient addition (Fig. 2.16), which may have contributed to size distribution resolution between nutrient addition treatments, meaning more small macroaggregates from the control were breaking down by August than small macroaggregates in soils with nutrient addition. In Figure 2.16, a graph of the ratio of free:occluded microaggregates is displayed. At the end of the season, the ratio of free:occluded microaggregates is >1 whereas it is lower at the beginning of the season. This means there are more free microaggregates than occluded microaggregates at the end of the season, whereas the opposite was true at the beginning of the season. Macroaggregate breakdown and turnover may have led to an increase in nitrogen concentration in free microaggregates in August because a portion may have been formerly occluded within macroaggregates (Six *et al*. 1999).

Section 2.4 Subsection 5: Potential linkages to other studies

Nitrogen limitations in tussock tundra (Weintraub and Schimel 2005) may have contributed to small macroaggregate turnover by the August sample collection date, based on an increase in the free:occluded microaggregate ratio (Fig. 2.16). According to the aggregate turnover and stabilization model of Six *et al.* (1999), aggregate formation is dependent on litter input and reformation around POM upon disruption. Low quality (Low N) litter may reduce aggregate formation because it is less susceptible to microbial decomposition. In their work at the same arctic site, Wallenstein *et al*. (2009) found that microbial enzyme activity declined over the growing season in control tussock tundra, but did not decline in soils where nitrogen was not as limiting as in tussock tundra.

The conclusions of Wallenstein *et al*. (2009) combined with the conceptual model of Six *et al*. (1999), taken together with my results, suggest that macroaggregates in control soils became N-limited, which resulted in a loss of small macroaggregate stability in control soils. Their breakdown led to a release of occluded microaggregates into the free microaggregate pool. The formerly occluded microaggregates contained greater concentrations of carbon and especially nitrogen than free micros, which resulted in a new free microaggregate pool of higher quality than earlier in the season. Microaggregate released from disrupted macroaggregates resulted in an increase in free microaggregate carbon content, which has also been observed in forested systems (He *et al*. 2008). Results in Chapter 3 (see percentage of iPOM C in free microaggregates portion of Fig. 3.5) may also provide evidence that microaggregates with enriched carbon present at the end of the season actually contain the carbon, and that this carbon is not simply an increase in fine light fraction organic matter outside the aggregate.

<u>Section 2.4 Subsection 6: Comparison to preliminary studies</u>

Ending the growing season with high quality microaggregates and SOM may result in the formation of new macroaggregates with greater stability than the ones measured in 2007. Aggregate dynamics during the 2006 growing season were measured as a preliminary study, and soils in the control increased in structure (Increased MWD) and SOM content over the growing season while the MWD of soils under nutrient addition increased somewhat, but less so than the control, while carbon content declined (Chapter One). In 2007, the same effect of nutrient addition over the growing season was not observed as it was in 2006. These results may imply that 2007 may have been a "rebuilding year", meaning there was no difference in soil carbon storage with nutrient addition, and that multi-year cycles of aggregate formation/breakdown may exist in these soils. Soils under nutrient addition were beginning to form macroaggregates that may have contained re-occluded microaggregates whereas control soils were undergoing macroaggregate disruption in August (Fig. 2.16). This release of high-quality microaggregates late in the season, which would serve as seeds of new macroaggregate formation (Angers *et al*. 1997) for the next growing season in control soils.

Section 2.4 Subsection 7: Effects of time under nutrient addition

No clear effects of time under nutrient addition emerged. If significant treatment effects were found, they were typically between control soils and soils with nutrient addition since 1989. One notable exception is figure 2.16, where soils with nutrient addition since 1996 differed from the control whereas soils with nutrient addition since 1989 did not. Soils with nutrient addition since 1996 often did not reflect an intermediate condition between the other two soils (e.g. Figs. 2.3 and 2.4). Often, soils with nutrient addition since 1996 would not differ from either the control or soils with nutrient addition since 1989. These results imply that the effect of long-term nutrient addition is not necessarily additive. However, soils with nutrient addition for the longest period of time (since 1989) tended to exhibit more differences from the control than soils with nutrient addition since 1996. Conversely, the soils with a shorter period of nutrient addition did not necessarily exhibit a treatment response that was intermediary between the control and soils with nutrient addition since 1989. In addition, it is unclear whether soils with nutrient addition since 1989 have reached a new steady state, nor is it clear when such a state would be reached.

Conclusions

Aggregate formation and disruption occurred during the course of the growing season in arctic soils. Nutrient addition affected aggregate size class distribution only in mid-June, which indicates that this is a dynamic period of aggregate formation and may be dependent on the microbial community and nitrogen availability. As the growing season progressed, there was a release of previously-occluded microaggregates upon macroaggregate disruption. Because occluded microaggregates tended to possess higher carbon and nitrogen contents than free microaggregates, once macroaggregates were disrupted, occluded microaggregates with a higher OM content than free microaggregates entered the free microaggregate pool, resulting in a free microaggregate pool with SOM quantities more similar to the occluded microaggregate pool. These results highlight the importance of multiple sample collection dates, which are necessary if we are to improve our understanding of factors driving SOM stabilization in Arctic soils.

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CHAPTER THREE

LIGHT FRACTION AND INTRA-AGGREGATE PARTICULATE ORGANIC MATTER SEPARATIONS FROM SMALL MACROAGGREGATES AND MICROAGGREGATES

Section 3.1. Introduction

Arctic soils contain a large proportion of soil organic matter (SOM) that is not chemically stabilized and is, therefore, potentially mineralizable. This SOM pool exists because cold, anoxic conditions restrict decomposition (Weintraub and Schimel 2003; reviewed in Chapter One). Light fraction organic matter, being a labile source of mineralizable carbon and nitrogen that is sensitive to land-use change (Cambardella and Elliott 1992; Whalen *et al*. 2000; He *et al*. 2007; Wagai *et al*. 2009), may be a component of this SOM pool. Tundra SOM is largely of plant origin (Weintraub and Schimel 2003), and due to low decomposition rates, a pool of slightly decomposed light fraction SOM should exist in these soils. Separating organic matter fractions through physical fractionation may help separate SOM with differing turnover times, particularly partially decomposed plant litter in the non-aggregate-associated light fraction from mineral-associated SOM and intra-aggregate particulate organic matter (iPOM) (Sollins *et al*. 1984; Whalen *et al*. 2000).

The focus of the previous chapter (Chapter 2) of this dissertation was on size distribution and temporal dynamics of water-stable soil aggregates as a means of physical stabilization of arctic SOM. The objective of the work contained within the current chapter was to separate free POM (light fraction) from previously separated aggregate fractions as well as from iPOM fractions contained within small macroaggregates and microaggregates (H4 from Chapter 1). Determining the size and dynamics of light fraction organic matter pools will aid in our understanding of how non-physically-protected organic matter is affected by nutrient addition.

Particulate organic matter (POM) can be classified as mineral-free POM and mineral-associated OM (Theodorou 1990). It may either be associated with aggregates as intra-aggregate particulate organic matter (iPOM), or free (non-aggregate-associated) POM (Six *et al*. 1998). Free POM is referred to as the Light Fraction (LF), and can be separated from the Heavy Fraction (HF), which includes mineral-associated OM and iPOM, through density separation. The light fraction is generally defined as a POM fraction with a density <1.85 g cm^{-3}, and is comprised largely of roots and other plant debris, hyphae, and charcoal (Spycher *et al*. 1983). Light Fraction organic matter is partially decomposed and has a C:N greater than the whole soil, which also contains mineral-associated organic matter and POM occluded within aggregates (Sollins *et al*. 1984; Tan *et al*. 2007), but lower than root C:N (Molloy and Speir 1977; Theodorou 1990), which indicates that some decomposition has occurred (Molloy *et al*. 1977). The light fraction pool has intermediate to fast turnover time (<5 years) that is slower than fresh litter, but faster than mineral-associated OM (Wander *et al*. 1994; Christensen 2001; Swanston *et al*. 2002; Yamashita *et al*. 2006).

The light fraction is dominated by plant carbohydrates whereas occluded OM is more decomposed and is more recalcitrant, containing a higher lignin and alkyl structure content, than the light fraction. (Golchin *et al*. 1994; Golchin *et al*. 1997). However, when separating light fraction and iPOM into coarse- and fine-sized components, Six *et al*. (1999a, 2001) found that fine light fraction was less labile than iPOM. The aggregate turnover model of Six *et al*. (1998) suggests that formerly occluded OM may re-enter the Free OM (light fraction) pool when aggregates break down. The end result is a mixture of light fraction and formerly occluded POM, but in general, the light fraction is dominated by labile, easily decomposed SOM (Theodorou 1990; Ashagrie *et al*. 2007; Tian *et al*. 2009, but see Swanston *et al*. 2002). Some light fraction may be formally occluded POM, but most consists of fungal biomass and root fragments in particular, and has been linked to root turnover in forest soils (Spycher *et al*. 1983).

Labile organic matter turnover dynamics are linked to the size of the light fraction pool, along with its carbon and nitrogen content (Janzen *et al*. 1992). Soil organic matter fractionation has been used to estimate the stability of carbon pools in grassland and cultivated soils. In a native grassland, mineral associated carbon constituted 60% of soil organic carbon, while the POM fraction made up the remaining 40% (Cambardella and Elliott 1992). Carbon content declined with increasing cultivation intensity, while the proportion of carbon associated with the mineral fraction increased and the POM fraction decreased. Based on these results, it is likely that the carbon fraction most affected by disturbance through cultiviation is POM-C, which is an organic matter fraction with an intermediate turnover time (Cambardella and Elliott 1992). These results were supported by the work of Six *et al*. (1998), who found that 47.5% of POM was lost as light fraction, which constituted 42% of the soil carbon lost upon cultivation, which is disproportionate to the proportion of total soil carbon that exists as light fraction. Elsewhere, Dalal and Mayer (1986) observed that light fraction carbon loss was 2-11 times greater than heavy fraction (mineral-associated) POM following cultivation; this loss may have been due to a lack of physical protection of light fraction carbon.

Carbon loss in temperate systems has been linked to a loss of light fraction organic matter (Six *et al*. 1998; Cambardella and Elliott 1992; Dalal and Mayer 1986). Because much of the low Arctic consists of tussock tundra, where mineral soil is overlain by a thick organic layer, it is likely that a substantial light fraction pool exists within this system. We do know that shifts in nutrient dynamics in the Arctic can result in a release in carbon from the system (Oechel *et al*. 1993; Mack *et al*. 2004); however, the dynamics of light fraction organic matter in the Arctic are not well-known. We do not know how much light fraction is incorporated into aggregates as iPOM, or the dynamics of occlusion within aggregates or release upon aggregate disruption. If light fraction organic matter is an organic matter fraction that is disproportionately susceptible to decomposition in the Arctic as it is in temperate areas (e.g. Six *et al*. 1998), then understanding the factors that lead to its occlusion within aggregates, existence outside aggregates, and substrate inputs will improve our understanding of soil carbon storage dynamics in the Arctic.

The general hypothesis for this chapter (first discussed in Chapter 1) is:

H4: *Particulate organic matter carbon content, nitrogen content, and location within the soil matrix as either light fraction or iPOM in arctic tundra are dynamic under natural conditions, responding to changes in organic matter inputs, microbial activity, soil aggregate turnover, and temperature.*

P4.1: Nutrient addition should have a lower impact on mineral-associated silt+clay carbon and nitrogen concentrations during the growing season than it would on POM fractions.

P4.2: Nutrient addition should alter light fraction carbon content dynamics. If new organic matter inputs increase due to active root decomposition, then the rate of material entering the light fraction pool will increase. At the same time, if the rate of light fraction decomposition increases with nutrient addition, then its contribution to the soil carbon pool may decrease by the end of the growing season.

P4.3: Nutrient addition should alter intra-aggregate particulate organic matter (iPOM) carbon content dynamics. If nutrient addition has resulted in shifts in the plant community, plant growth rates, and decomposition, then material being incorporated into aggregates (iPOM) could be reduced by the end of the growing season through a decrease in iPOM pool size (due to increased macroaggregate turnover), or increased through increased root inputs and subsequent aggregate formation as the growing season progresses.

P4.4: Nutrient should alter light fraction nitrogen storage dynamics. Increased root growth due to nutrient addition provides new material for microbial decomposition and subsequent incorporation into the SOM pool. At the same time, nutrient addition stimulates decomposition. Therefore, the proportion of aggregate and whole soil nitrogen that the light fraction comprises should increase over the growing season if the light fraction is being decomposed at a greater rate in soils with nutrient addition than control soils.

P4.5: If both the rate of light fraction formation and decomposition are increasing with nutrient addition, then over the course of the growing season, the light fraction C:N would decrease if decomposition exceeds formation, and would increase if formation exceeds decomposition. A lower C:N results from carbon being lost as carbon dioxide while nitrogen is conserved within the system.

P4.6: Root biomass dynamics are affected by nutrient addition. If root biomass increases, then there will be a seasonal increase in the amount of fresh, labile organic matter entering the SOM pool, comprising the light fraction and iPOM fractions.

Section 3.2. Procedures

Section 3.2 Subsection 1: Density Separation Procedure

The initial separation of aggregate size classes and soil collection from the field is described in Chapter 2. Of those aggregates, density separations were completed for small macroaggregates (250-2000 μm) and free microaggregates (53-250 μm). Sodium polytungstate (SPT) was used to create a high-density solution used to separate light fraction from heavy fractions using the method of Six *et al*. (1998). Using SPT allows carbon and nitrogen measurements to later be made of the light fraction as well as the heavy fraction because SPT has very little impact on the carbon and nitrogen content of the sample (Six *et al*.1999b).

For each LF/HF separation (Fig. 1.1 in Chapter 1), prior to density separation, aggregate fractions were dried (60ºC) then cooled to room temperature in a dessicator. Subsamples (5 g) were added to a 50 ml centrifuge tube containing 25 ml of sodium polytungstate (SPT) at a density of 1.85g cm^{-3}. Samples were gently mixed in order to avoid aggregate disruption, the centrifuge tubes filled to 40 ml with SPT, and placed under vacuum (138 kPa) to remove air trapped within aggregates. Afterward, centrifuge tubes containing samples were centrifuged (1250 g) for 60 min to separate light from heavy fractions. Floating material, which comprises the light fraction, was aspirated onto a 20μm nylon filter, where the SPT was rinsed off with water. Samples were dried, weighed, and analyzed for carbon and nitrogen content using a Carlo Erba (Milan, Italy). The silt and clay fraction was separated from sand by dispersing the heavy fraction with sodium hexametaphosphate (0.5% solution by weight), then passing the dispersed fraction through a 53 μm sieve. The carbon and nitrogen content of the <53μm fraction were quantified by dry combustion using a Leco TruSpec CN analyzer (Leco Corporation, St. Joseph, Michigan).

Sodium polytungstate was recycled using the method of Six *et al*. (1999b). Carbon and nitrogen content of the recycled SPT did not exceed the limits of accuracy of the Leco TruSpec CN analyzer (Leco Corporation, St. Joseph, MI) used for measuring carbon and nitrogen content of other aggregate fractions.

<u>*Section 3.2 Subsection 2: Statistical methods*</u>

Field plots were described in Chapter Two (n=4). Control soils in block 1 from samples collected in late June were not used in analysis because they were organic, and not mineral, soils. All data were analyzed using SAS statistics software for analysis of variance (SAS Institute, 2003). Sub-samples were nested within treatment plots within each block. Block and block*treatment interactions were treated as random effects. Kenward-Roger degrees of freedom were used because the number of subsamples per plot varied from 1-3. Mean separations were tested using Tukey's honestly significant difference. Because of high natural heterogeneity in arctic soils, effects were considered to be significant at $p \leq 0.10$. Data were log transformed to meet assumptions of normality.

Section 3.3. Results

Here the results for carbon and nitrogen data from silt+clay (<53µm)-associated organic matter, small macroaggregate and microaggregate light fraction, carbon data from small macroaggregate and microaggregate iPOM, and whole soil live root biomass are presented. Data is first presented on carbon and nitrogen associated with the silt and clay (<53µm) fraction. Light fraction and iPOM carbon are then presented, followed by light fraction nitrogen and C:N. It was not possible to calculate iPOM nitrogen content due to method constraints; therefore, nitrogen and C:N are presented for the light fraction POM only.

Section 3.3 Subsection 1: Silt+Clay-associated Carbon and Nitrogen (P4.1)

Silt+clay associated carbon and nitrogen comprised the dominant small macroaggregate carbon (Fig. 3.1) and nitrogen (Fig. 3.2) fractions. There were no significant nutrient addition effects. It is not unexpected that there were no treatment effects on SOM associated with silt+clay. This material represents a relatively stable SOM fraction, whereas the light fraction and iPOM fractions can be comprised of labile SOM.

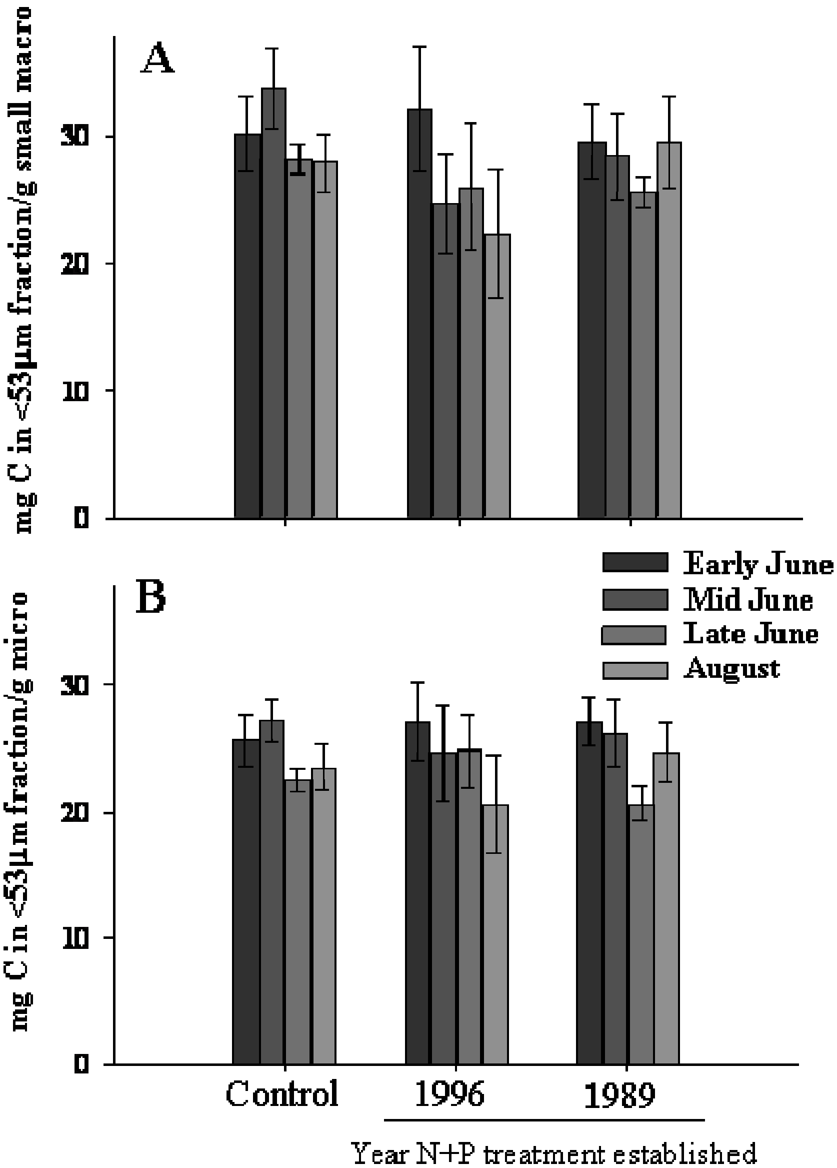

Figure 3.1. Silt and clay (<53μm) carbon content (mg C/g aggregate) within small macroaggregates (A) and microaggregates (B). Bars represent mean ± standard error. No significant effects.

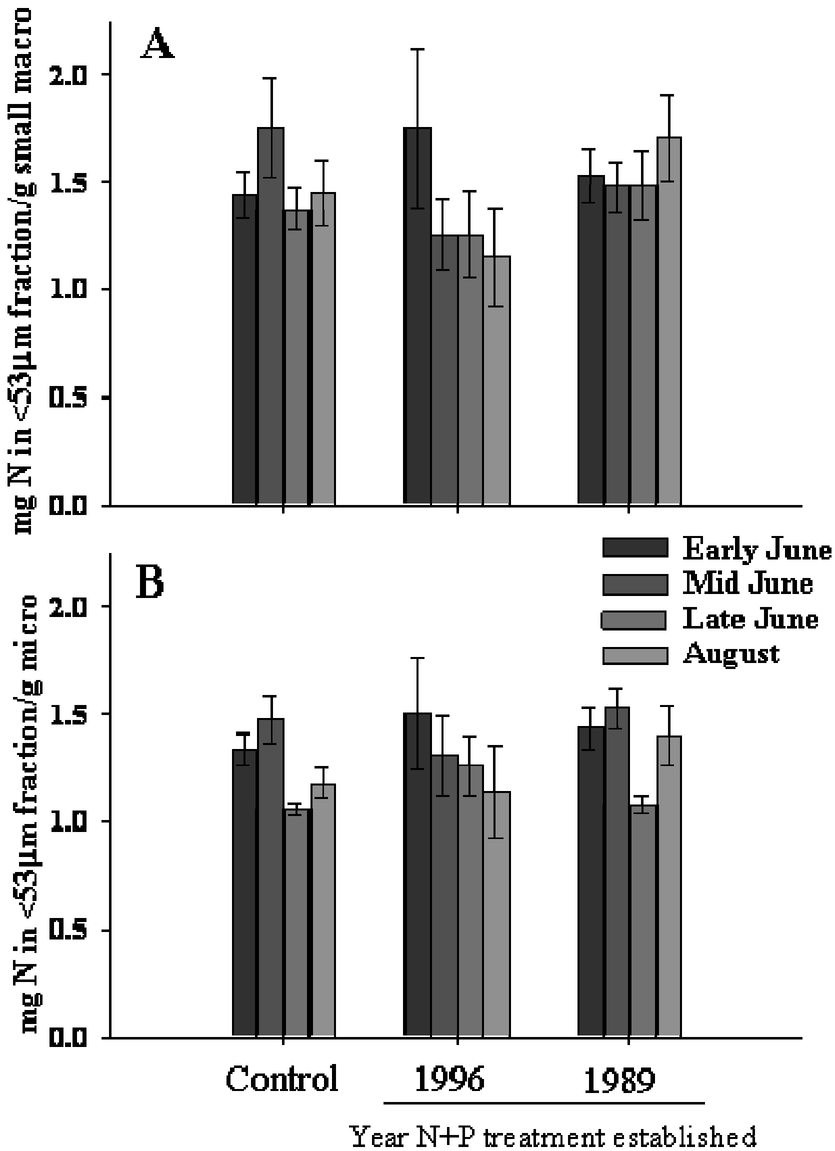

Figure 3.2. Silt and clay (<53μm) nitrogen content (mg N/g aggregate) within small macroaggregates (A) and microaggregates (B). Bars represent mean ± standard error. No significant effects.

Section 3.3 Subsection 2: Light Fraction and iPOM Carbon (P4.2 and P4.3)

This section focuses on light fraction and iPOM carbon, with the data presented from several different perspectives:

- Figure 3.3 displays the percentage of small macroaggregate and microaggregate carbon as light fraction/iPOM carbon (% of aggregate carbon), which corrects for differences in the total carbon concentration in each aggregate and allows comparisons of the relative contribution of light fraction/iPOM to total aggregate carbon to be made.
- Figure 3.4 displays the amount of light fraction/iPOM C from small macroaggregates and microaggregates on a whole soil basis (mg C/g whole soil), which provides perspective on the actual size of each POM carbon fraction.
- Figure 3.5 displays the percentage of whole soil carbon as small macroaggregate/microaggregate light fraction/iPOM, which corrects for differences in total carbon and allows comparisons of the relative contribution of light fraction/iPOM to be made.
- Figure 3.6 displays the percentage of whole soil carbon as combined small macroaggregate and microaggregate light fraction carbon, giving a perspective of nearly the total light fraction in these soils. Light fraction associated with the large macroaggregates is not presented because in most instances there was not enough material for light fraction separation, but also because 2000 μm is often given as the upper limit of the size of particulate organic matter in soil (Six *et al.* 2002).

The percentage of small macroaggregate and microaggregate carbon as iPOM-C was significantly affected by nutrient addition (Fig. 3.3). In early June, there were no differences in iPOM C, but in mid-June, the percentage of small macroaggregate C as iPOM-C was greater in soils with nutrient addition since 1996 than the control. The percentage of microaggregate carbon as iPOM-C was similar to small macroaggregate iPOM-C, except in late June, soils with nutrient addition since 1989 had a greater percent of microaggregate carbon associated with iPOM than soils with nutrient addition since 1996 and the control. By the August sample date, there were no significant treatment differences in iPOM-C allocation within both microaggregates and macroaggregates.

The percentage of small macroaggregate and microaggregate carbon as light fraction carbon did not differ significantly with nutrient addition (Fig. 3.3). However, the patterns of carbon allocation over the growing season were similar to the iPOM. In both iPOM and light fraction, the control soils did not seem to vary between the beginning and end of the growing season. Soils with nutrient addition, on the other hand, appeared to accumulate iPOM and light fraction carbon, having a larger percentage of the small macroaggregate and microaggregate carbon associated with iPOM and light fraction at the end of the season than the beginning.

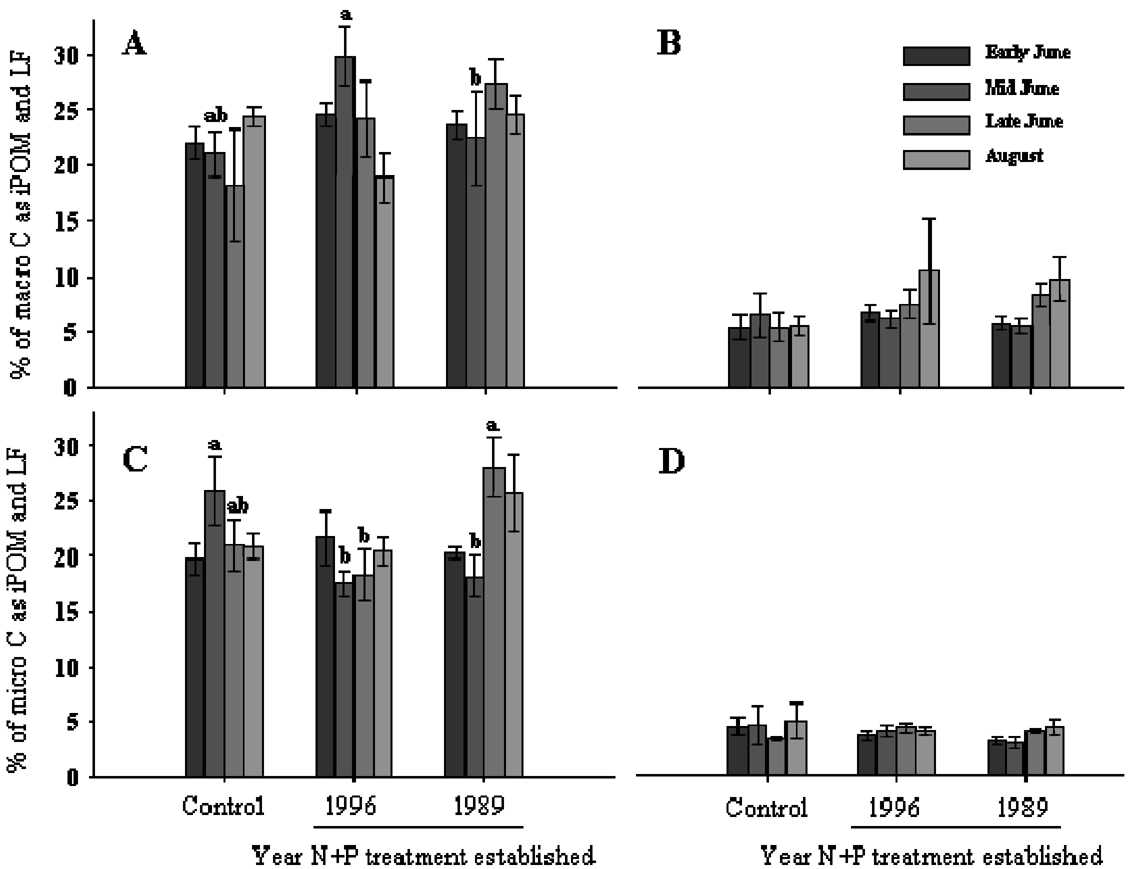

Figure 3.3. Percent of small macroaggregate C as iPOM-C (A) and LF-C (B), and microaggregate C as iPOM-C (C) and LF-C (D). Bars represent mean ± standard error. Lowercase letters indicate significant differences between nutrient addition treatments on a sample date ($p \leq 0.10$).

On a whole soil basis (mg C/g light fraction or iPOM), there were no significant treatment effects (Fig. 3.4). In general, there was more small macroaggregate light fraction and iPOM C/ g of whole soil than microaggregate light fraction and iPOM C. This difference was due to soils containing more small macroaggregates than microaggregates in all cases (Chapter 2).

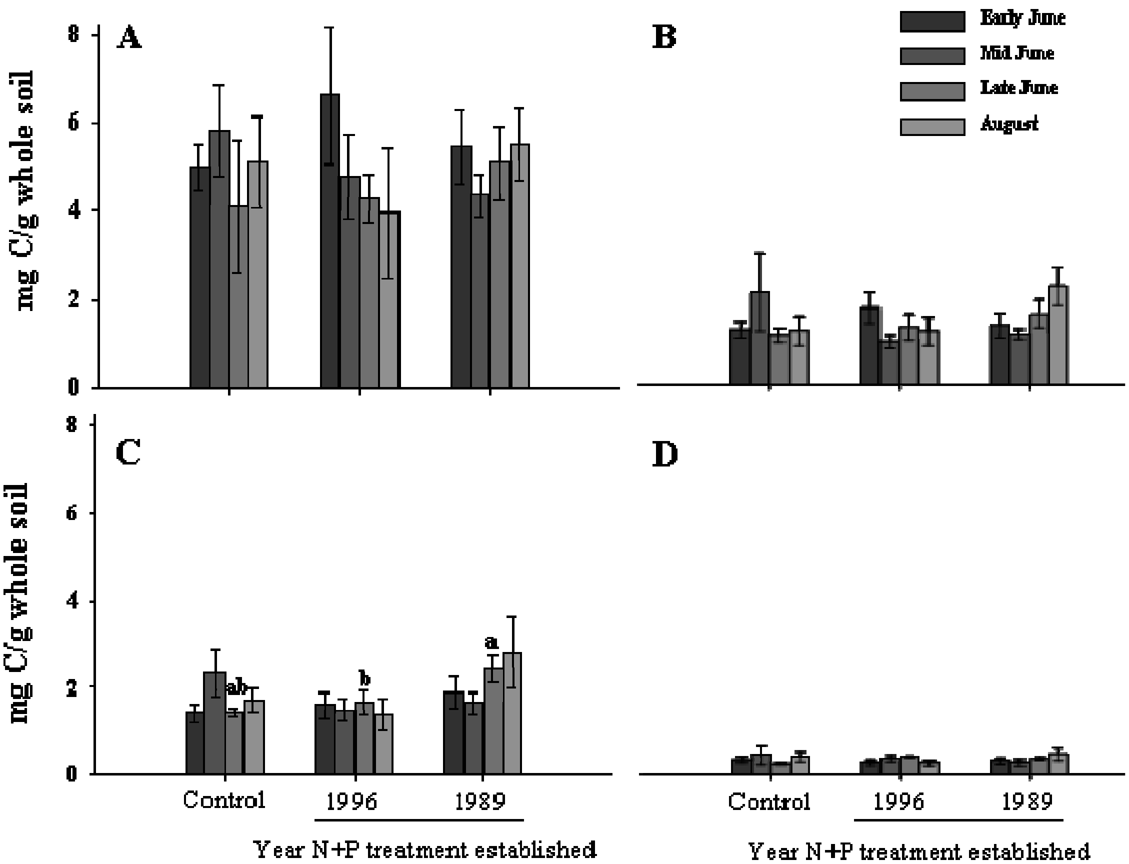

Figure 3.4. Whole soil C (mg C/g whole soil) from small macroaggregate iPOM-C (A) and LF-C (B), and microaggregate iPOM-C (C) and LF-C (D). Bars represent mean ± standard error. Lowercase letters indicate significant differences between nutrient addition treatments on a sample date ($p \le 0.10$).

The percentage of whole soil carbon as light fraction and iPOM carbon was significantly affected by nutrient addition in later sample dates (Figure 3.5). In small macroaggregates, the percentage of whole soil carbon as light fraction carbon was significantly greater ($p \le 0.05$) in soils with nutrient addition since 1989 than the control. Small macroaggregate iPOM C exhibited similar patterns, though there were no significant differences. However, in microaggregates, the percentage of whole soil

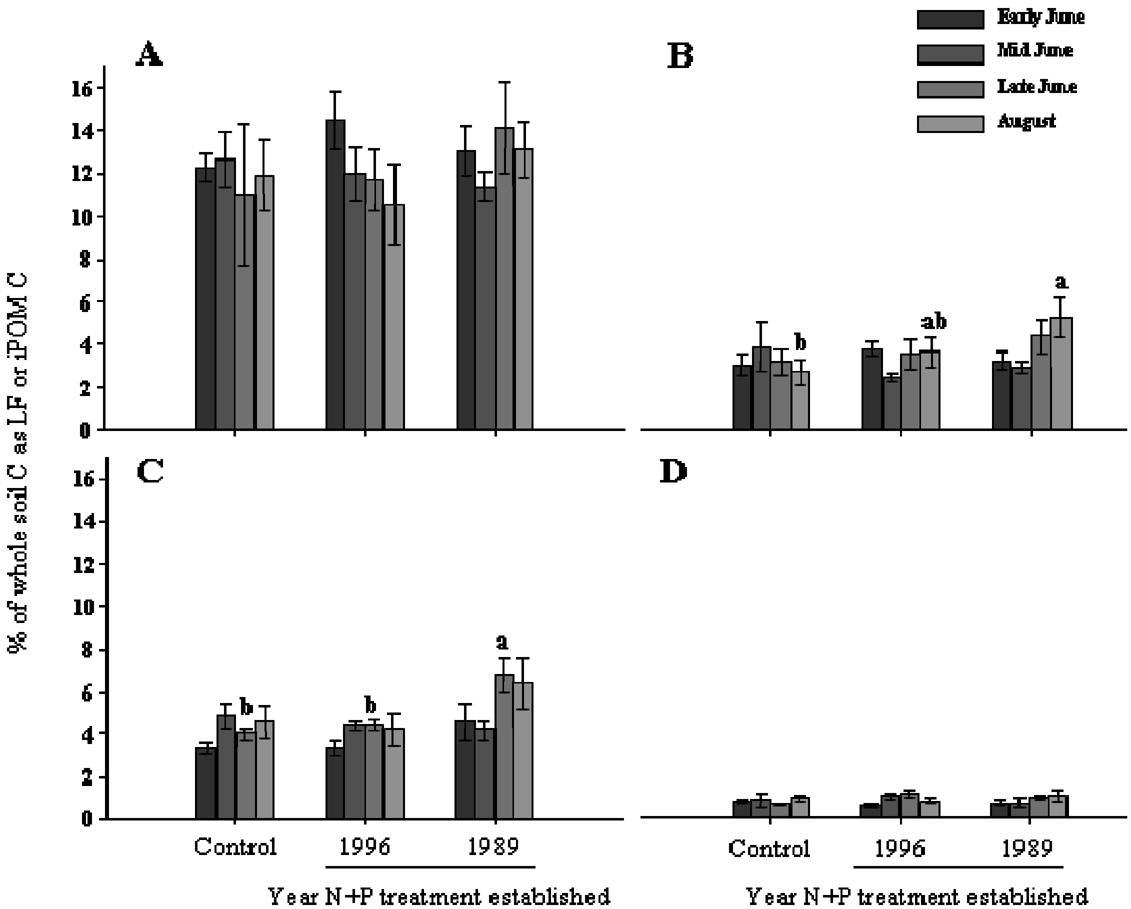

Figure 3.5. Percent of whole soil carbon as small macroaggregate iPOM (A) and light fraction (B), and microaggregate iPOM (C) and light fraction (D). Bars represent mean ± standard error. Lowercase letters indicate significant differences between nutrient addition treatments on a sample date ($p \leq 0.05$).

carbon as microaggregate iPOM carbon was greater in soils with nutrient addition since 1989 than the control and soils with nutrient addition since 1996. There were no significant differences in microaggregate light fraction carbon.

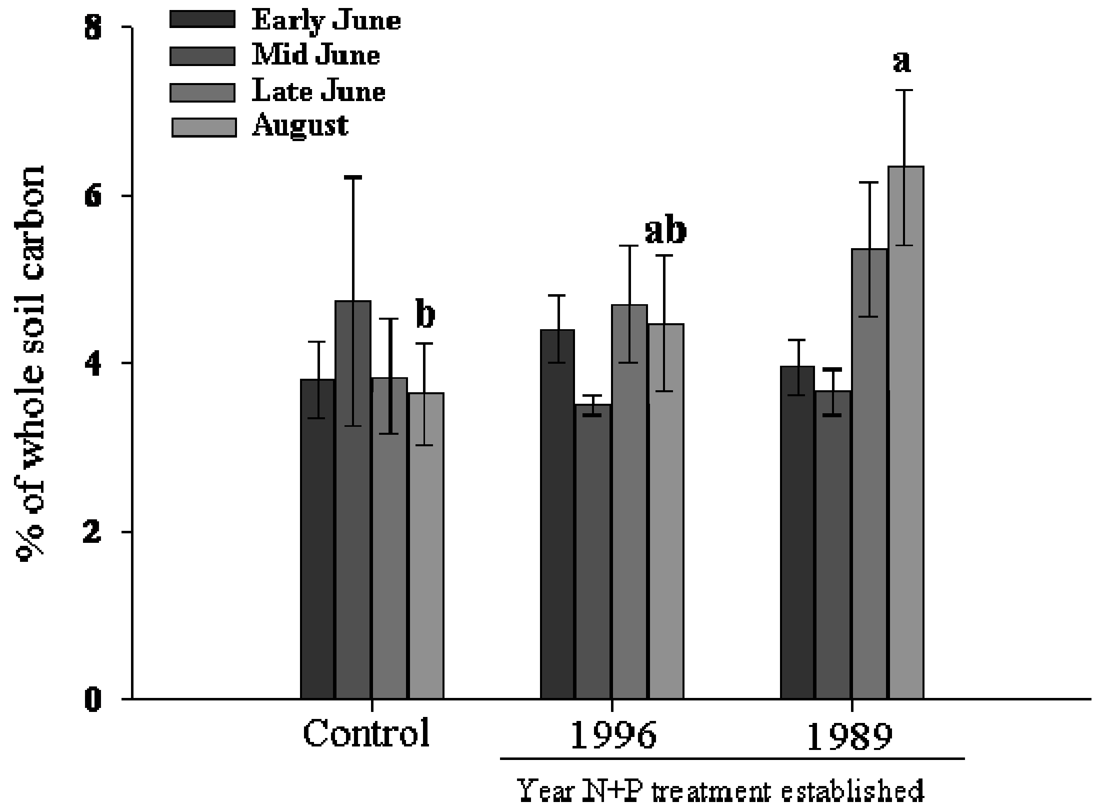

Figure 3.6. Percent of whole soil carbon as combined small macroaggregate and microaggregate light fraction carbon. Bars represent mean ± standard error. Lowercase letters indicate significant differences between nutrient addition treatments on a sample date ($p \leq 0.075$).

In Figure 3.6, the percent of whole soil carbon as light fraction carbon is shown for small macroaggregate and microaggregate light fraction added together. Soils with nutrient addition since 1989 had a significantly larger ($p \leq 0.075$) percentage of the whole soil carbon allocated to the light fraction than the control. The only missing light fraction would come from the large macroaggregate light fraction, but because large macroaggregates comprise a small portion of the whole soil, it was not possible to fractionate it (Chapter 2).

Section 3.3 Subsection 3: Light Fraction Nitrogen (P4.4)

This section focuses on light fraction nitrogen content, with the data presented from several different perspectives in a manner similar to the carbon data:

- Figure 3.7 displays the percentage of small macroaggregate and microaggregate nitrogen as light fraction nitrogen (% of aggregate nitrogen).
- Figure 3.8 displays the amount of light fraction nitrogen from small macroaggregates and microaggregates on a whole soil basis (mg N/g whole soil), which provides perspective on the actual size of each POM nitrogen fraction.
- Figure 3.9 displays the percentage of whole soil nitrogen as small macroaggregate/microaggregate light fraction, which corrects for differences in total nitrogen and allows comparisons of the relative contribution of light fraction to be made.
- Figure 3.10 displays the percentage of whole soil nitrogen as combined small macroaggregate and microaggregate light fraction nitrogen, giving a perspective of nearly the total light fraction in these soils.

The percentage of total aggregate nitrogen comprised as LF-N was determined in the small macroaggregates (250-2000 μm) and microaggregates (53-250 μm). As a seasonal average, the LF of soils with nutrient addition contained a greater percentage of aggregate nitrogen than the control. In August, the percentage of aggregate nitrogen from the small macroaggregate LF was significantly greater in soils with nutrient addition since 1989 than the control (Fig. 3.7). Microaggregate LF-N exhibited similar trends, though none differed significantly from one another.

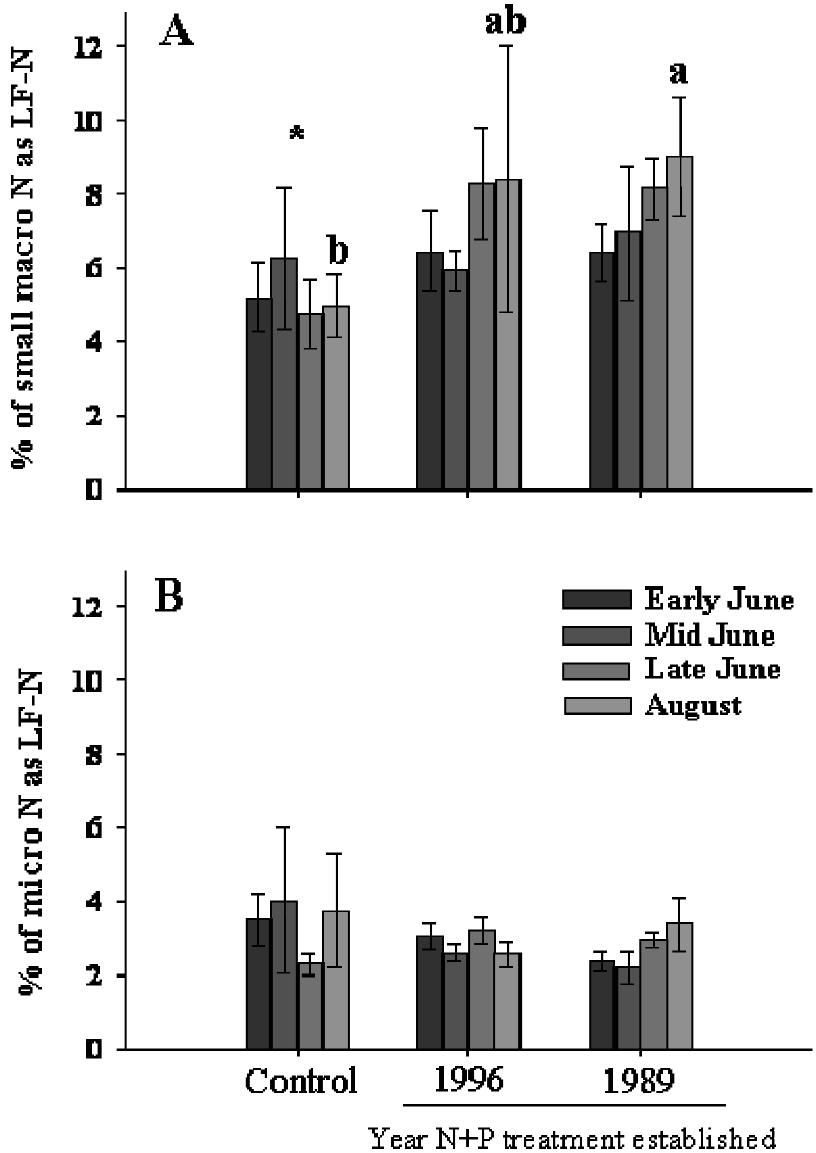

Figure 3.7. Percent of small macroaggregate (A) and microaggregate (B) nitrogen as light fraction N. Bars represent mean ± standard error. Lowercase letters indicate significant differences between nutrient addition treatments on a sample date ($p \leq 0.075$). Asterisk indicates seasonal average difference from other N+P treatments ($p \leq 0.10$).

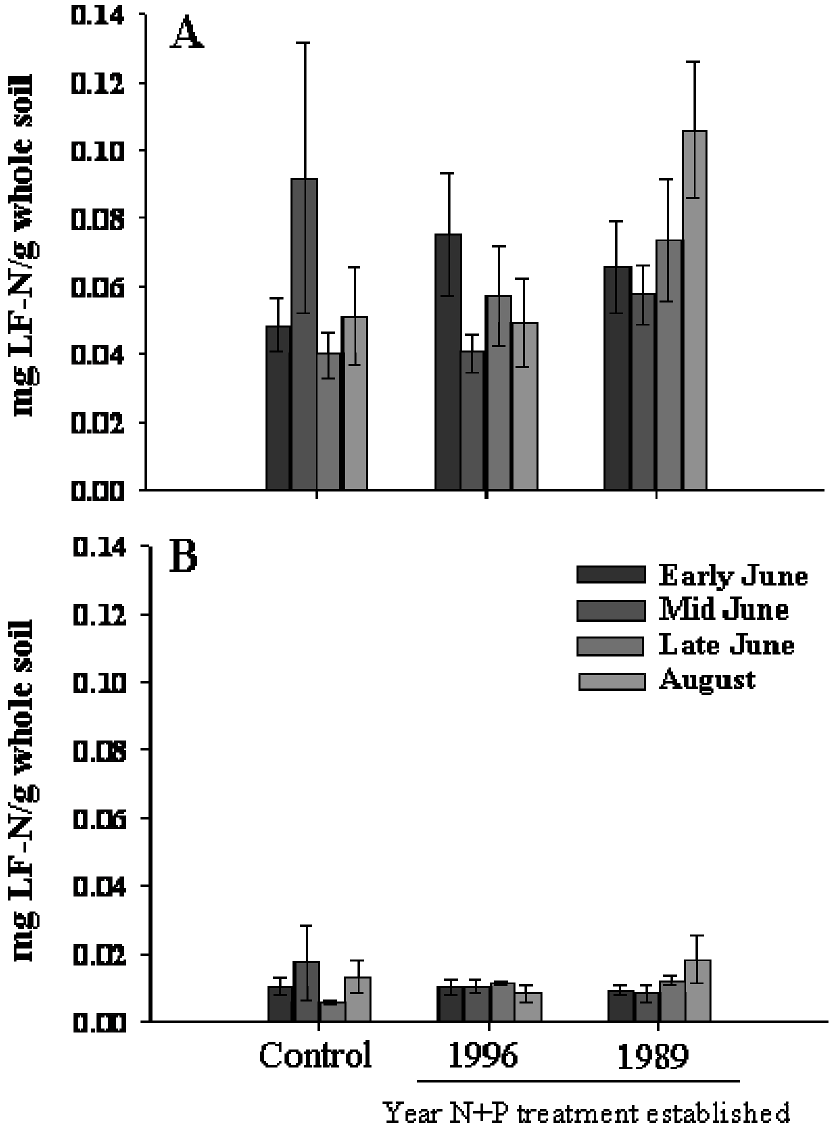

Figure 3.8. Whole soil light fraction nitrogen (mg LF-N/g soil) from (A) small macroaggregates and (B) microaggregates. Bars represent mean ± standard error. No significant effects.

Total nitrogen content of the light fraction was determined, but it was not possible to do so for iPOM due to low nitrogen content combined with the large error associated with solving nitrogen content by difference (Total N – LF-N - <53 μm N = iPOM-N), whereas total aggregate and light fraction nitrogen contents were directly measured. On a whole soil basis, LF-N increased (but not significantly) over the growing season in soils with nutrient addition since 1989 (Fig. 3.8).

The percentage of total soil nitrogen as light fraction nitrogen was affected by nutrient addition (Fig. 3.9). In small macroaggregates, the percent of total nitrogen as light fraction nitrogen increased over the growing season in soils with nutrient addition since 1989, and by August, was almost twice as much as the control ($p \leq 0.05$). A similar pattern was observed in microaggregates, except that in late June, soils with nutrient addition since 1996 contained a significantly greater percentage of soil nitrogen in the light fraction than the control ($p \leq 0.10$).

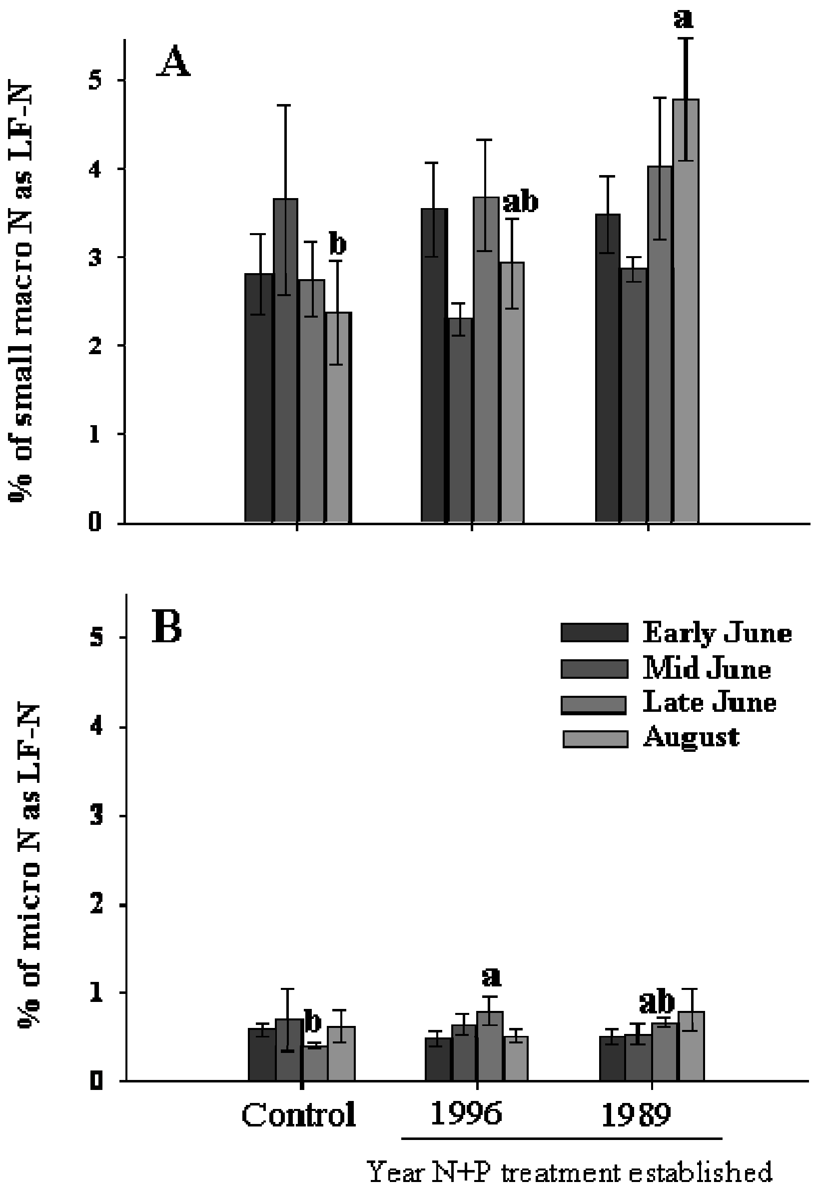

Figure 3.9. Percent of whole soil nitrogen as small macroaggregate (A) and microaggregate (B) light fraction N. Bars represent mean ± standard error. Lowercase letters indicate significant differences between nutrient addition treatments on a sample date ($p \leq 0.05$) for small macro (A) LF-N and ($p \leq 0.10$) for micro (B) LF-N).

Soils with nutrient addition since 1989 contained a significantly greater percentage of nitrogen in the light fraction by the end of the growing season ($p \leq 0.05$) (Figure 3.10). No clear trend was identifiable in soils with nutrient addition since 1996.

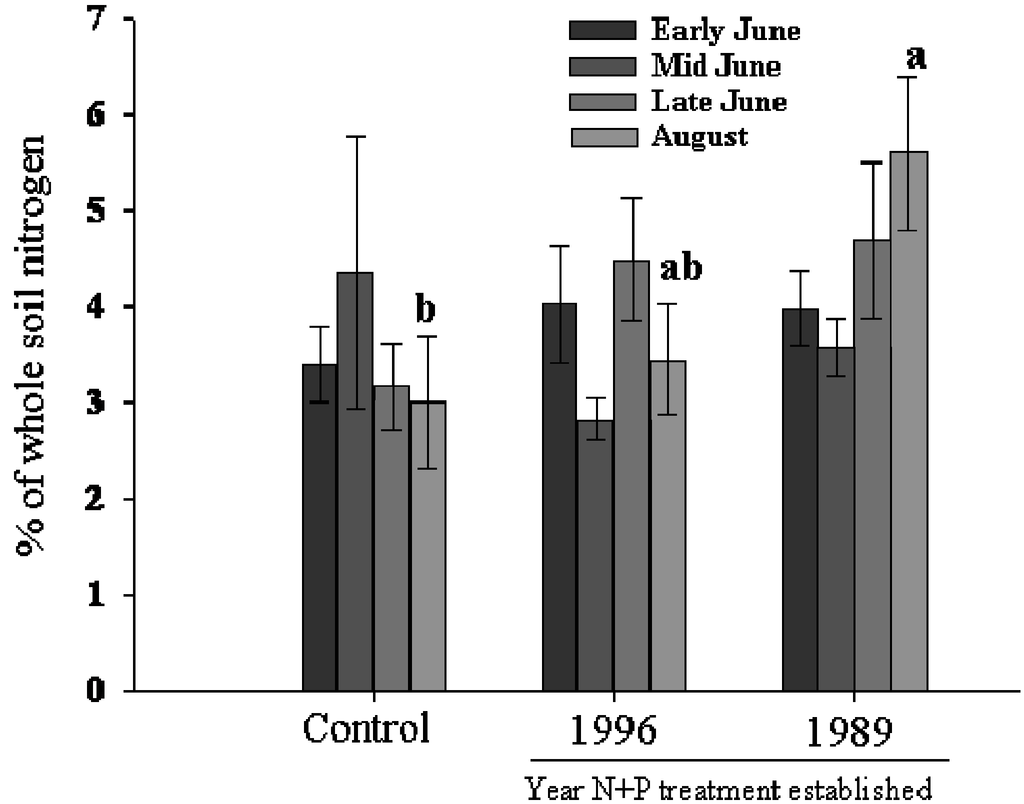

Figure 3.10. Percent of whole soil nitrogen as combined small macroaggregate and microaggregate light fraction nitrogen. Bars represent mean ± standard error. Lowercase letters indicate significant differences between nutrient addition treatments on a sample date ($p \leq 0.05$).

<u>*Section 3.3 Subsection 4: Light Fraction Carbon:Nitrogen (P4.5)*</u>

Light fraction C:N was lower in small macroaggregates than microaggregates (Fig 3.11, $p \leq 0.0001$). The C:N of the small macroaggregate LF was lower in soils with nutrient addition since 1989 than the control and soils with nutrient addition since 1996 ($p \leq 0.05$) as well as being lower than the control for three sampling dates and soils with nutrient addition since 1996 for two sampling dates ($p \leq 0.05$). In August, the C:N of the

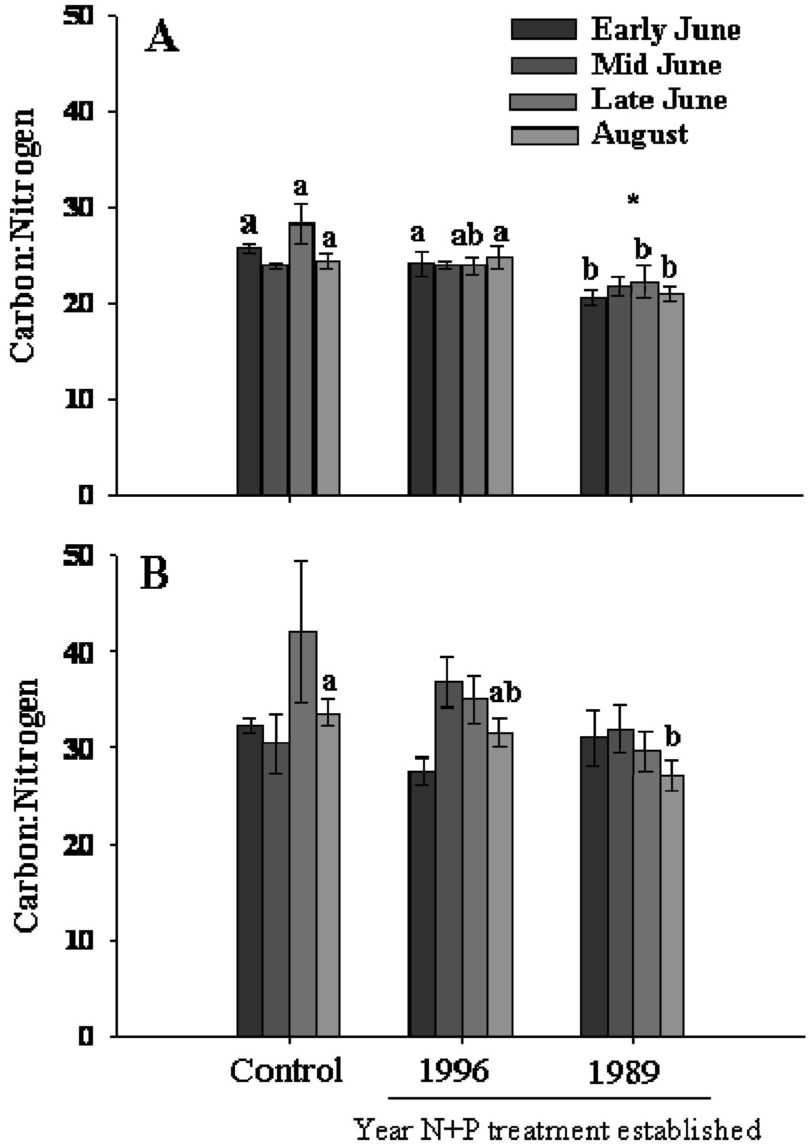

Figure 3.11. Carbon:Nitrogen ratio of small macroaggregate (A) and microaggregate (B) light fraction. Bars represent mean ± standard error. Lowercase letters indicate significant differences between nutrient addition treatments on a sample date ($p \leq 0.05$). Asterisk indicates seasonal average difference from other N+P treatments ($p \leq 0.05$).

microaggregate LF was significantly lower in soils with nutrient addition since 1989 than the control ($p \leq 0.05$).

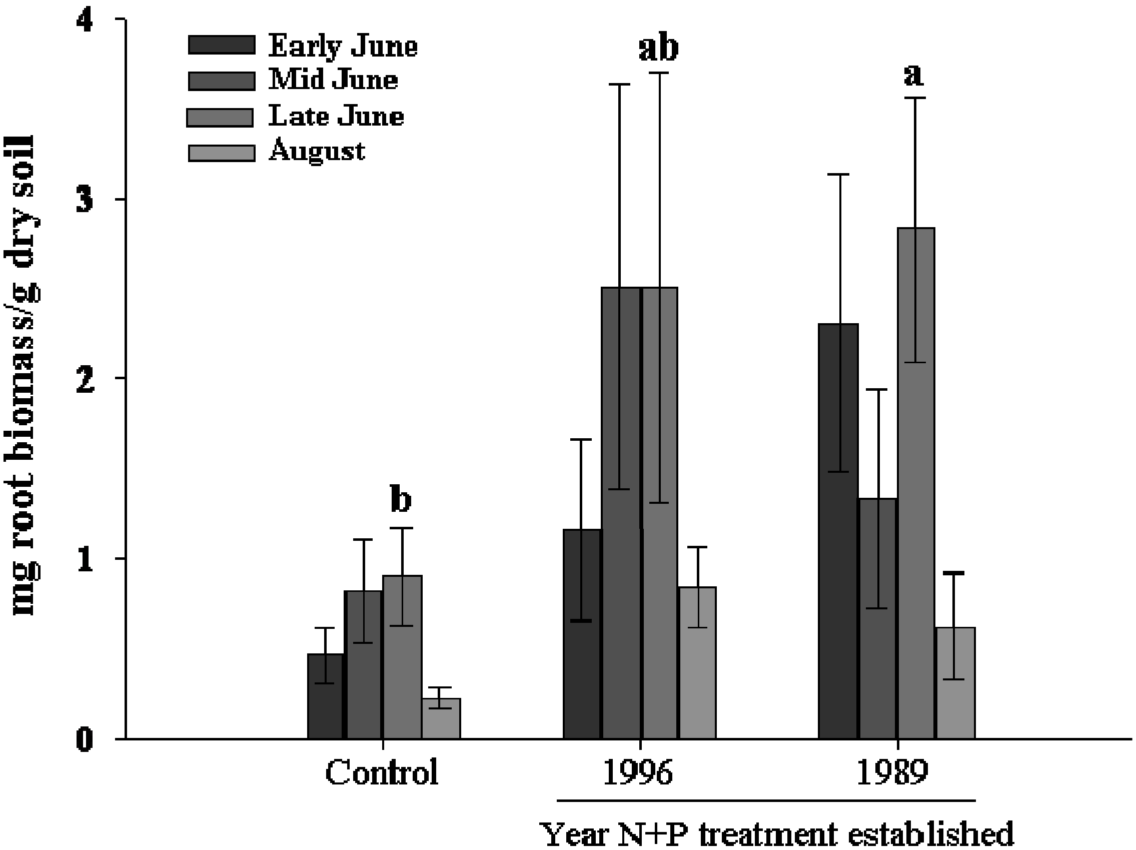

Figure 3.12. Live root biomass (mg roots/g whole soil). Bars represent mean ± standard error. Lowercase letters indicate significant differences between nutrient addition treatments on a sample date ($p \leq 0.10$). Mid and Late June sample dates were greater than August root biomass across all treatments ($p \leq 0.05$).

<u>*Section 3.3 Subsection 5: Mineral Soil Live Root Biomass (P4.6)*</u>

Whole mineral soil live root biomass (Fig. 3.12) increased in all plots in mid-late June, then declined by August ($p \leq 0.05$). In mid and late June, soils with nutrient addition since 1989 had significantly greater root biomass than control soils ($p \leq 0.10$). By August, the root biomass declined in all treatments, but most notably in soils with nutrient addition.

Section 3.4. Discussion

The research objective of this chapter was to separate Light Fraction and iPOM and mineral-associated SOM fraction from one another in small macroaggregates and free microaggregates. I hypothesized that POM fractions will be affected temporally and that nutrient addition will reduce decomposition constraints of the light fraction. Root biomass increased, coupled with increases in the proportion of soil carbon as particulate organic matter carbon over the growing season. Light fraction quality increased, which may indicate increased decomposition with nutrient addition.

Silt+clay-associated SOM (Prediction 4.1) was the dominant organic matter component in small macroaggregates and microaggregates. Other studies have found that the heavy fraction contains the bulk of SOM. Diochon and Kellman (2009) determined that in a forest soil, at least 70% of SOM was found in the heavy fraction. This pool is more recalcitrant than other SOM pools, particularly the light fraction. Mineral-associated SOM tends to be resistant to decomposition and has a longer turnover time than light fraction and iPOM (Theodorou 1990; Yamashita *et al*. 2006; Tian *et al*. 2009). The particulate organic matter fractions, iPOM and light fraction, are labile SOM fractions that differ from each other in that iPOM is physically protected within aggregate structure whereas light fraction is not (Christensen 2001). Whereas mineral-associated SOM is partially protected from decomposition and environmental change, the light fraction is a labile carbon pool that is sensitive to environmental change (Cambardella and Elliott 1992).

New light fraction would have more carbohydrates than occluded POM, which though being partially decomposed through aggregate formation processes would be older and have a lower carbohydrate content. However, if light fraction persists and is being decomposed and not incorporated into aggregates, than carbohydrates would be lost, leaving lignin and other resistant compounds behind (Six *et al*. 2001). The relative stability of light fraction vs. iPOM is highly dependent on both input (for light fraction) and aggregate turnover rates (for iPOM). If aggregates quickly form around POM, then the stability of Arctic POM would be similar to Six *et al*. (2001). If input rates are high, then it is likely that light fraction would be younger than iPOM and therefore more labile (Golchin *et al.* 1994, 1997).

Golchin *et al*. (1994) used sodium polytungstate at a density of 1.6 g-cm^{-3} whereas the sodium polytungstate used by Six *et al*. (1999, 2001) was 1.85 g-cm^{-3}, which is the same density used for the present study. It is likely that some of the more recalcitrant POM that Six *et al*. (1999, 2001) would have floated off would still have remained in the heavy fraction separated by Golchin *et al*. (1994), given that the most labile light fraction is at a lower density than the more recalcitrant light fraction.

In both small macroaggregates and microaggregates, the light fraction (Prediction 4.2) contained a disproportionate amount of carbon in relation to the mass of the fraction (Whalen *et al*. 2000), which has been commonly observed elsewhere. Tan *et al.* (2007) observed that of the small macroaggregate carbon, 5-10% was located in the light fraction, and 3-5% of microaggregate carbon was located in the light fraction. The light fraction mass was much lower than the heavy fraction, but on an equivalent measure (g C/kg fraction), contained 4.3-5.3 times more carbon than the heavy fraction (Tan *et al*. 2007).

The total Arctic SOM pool contains light fraction OM, which is largely comprised of plant material (Spycher *et al*. 1983; Weintraub and Schimel 2003). High light fraction C:N ratios (Prediction 4.5) indicate that this fraction is a younger fraction that has not decomposed as much as the total SOM pool, whose C:N is similar to the heavy fraction C:N (Turchenek and Oades 1979; Molloy and Speir 1977). However, the aggregate turnover model of Six *et al*. (1999a) suggests that iPOM may reenter the Free OM (light fraction) pool when aggregates break down. The end result is a mixture of LF and formerly occluded POM, but in general, the LF is dominated by labile, easily decomposed SOM, and may be a product of root turnover.

In mid and late June, an increase in root biomass was observed (Prediction 4.6), especially in soils with nutrient addition. However, root biomass then declined by over 80% from the late June root biomass measurements in soils with nutrient addition since 1989. This decline in root biomass in August occurred at the same time an increase in the proportion of SOM as light fraction and iPOM was observed, which indicates an increase in root turnover and incorporation into the light fraction and iPOM. Partial root decomposition has been found to be an important aspect of aggregate stabilization (Gale *et al*. 2000b; Kong and Six 2010). Similar light fraction and iPOM results have been observed elsewhere. Six *et al*. (1999a) suggested that iPOM is a young organic matter fraction that serves as a site for new macroaggregate formation. In a forested system, light fraction material increased by 50-100% from early spring to summer, peaking in the fall. (Spycher *et al*. 1983). Studies in tussock grasslands (non-arctic) have presented observations of larger proportions of SOM carbon and nitrogen located in the light fraction (Molloy and Speir 1977) than what was observed for this study. Others in native vegetation and cultivated systems have found similar results. Six *et al*. (1999a) found light fraction carbon content in soils from native vegetation in Ohio, Michigan, and Kentucky, and from no-till soils in Ohio and Nebraska that were similar to the soils that were analyzed for the present study.

The light fraction undergoes large seasonal fluctuations and is a labile source of carbon in forest systems (Spycher *et al*. 1983), cultivated systems (Six *et al*. 1998), and grasslands (Molloy and Speir 1977). The observed loss of mineral soil root biomass over the course of the growing season may indicate that the root growth rate is slower than the rate of decomposition. However the proportion of SOM as light fraction was greatest in soils with nutrient addition, which also had the lowest C:N ratio, indicating higher quality than control soils. There are two rates, light fraction decomposition and root growth (and subsequent fragmentation), that need to be considered alongside each other. Based on light fraction quantity, light fraction C:N, and root biomass data, the rates of both light fraction decomposition and root growth increased. Increased root growth provided an organic matter source for more light fraction. A larger light fraction pool was subjected to an increased decomposition rate, which resulted in a lower C:N.

The increase in the C:N ratio in microaggregate light fraction in mid-to-late June suggests that the peak belowground input relative to decomposition occurs at this time during the growing season (Fig. 3.11). The C:N ratio decreases by August. In the free microaggregates, the C:N is less at the end of the season than the beginning only for soils with nutrient addition since 1989, which may indicate that the carbon in these soils is turning over faster than the other soils. If the C:N is greater at the end of the season than the beginning, then the rate of input carbon is greater than the rate of output carbon.

Neff *et al*. (2002), observed an increase in light fraction decomposition with nitrogen addition, while Gregorich *et al*. (1997) observed an increase in the size of the light fraction pool with nutrient addition. The results of the present study may suggest a combination of the results of Gregorich *et al*. (1997) and Neff *et al*. (2002) may exist in the Arctic. Increased light fraction quantity with nutrient addition suggests increased root growth and fragmentation, while lower C:N indicates increased light fraction decomposition. Therefore, nutrient addition may stimulate both plant and microbial activity. At the same time, the observed increase in iPOM carbon (Fig. 3.4) would suggest that some of the light fraction is being incorporated into aggregates. Aggregate stabilization has been linked with higher root-derived iPOM content than in unstable aggregates (Gale *et al*. 2000a; Kong and Six 2010).

Microbial activity does not appear to be controlled by organic matter quantity to the extent that it is in temperate regions (Weintraub and Schimel 2003). Despite an increase in light fraction, no increase in aggregate formation was observed. In soils with nutrient addition since 1989, the proportion of aggregate carbon as iPOM tended to increase, but the amount of carbon as light fraction also increased and was not incorporated into aggregate structure. There was more light fraction and iPOM, but not more aggregates. The heavy fraction tends to be a source of mineral nitrogen while the light fraction is often a sink, and may be linked with microbial nitrogen immobilization (Whalen *et al*. 2000). The heavy fraction C:N ratios I measured tended to be greater than 20:1. Macroaggregate light fraction C:N was greater than the heavy fraction, but the C:N values of both were similar to each other. Microaggregate LF C:N's were generally >30:1. A decrease in LF C:N with nutrient addition is insufficient to increase decomposition, but because this fraction is not protected within aggregates, it lacks physical protection and may decompose under more favorable conditions, including lower limitations on nitrogen availability, which may affect microbial activity.

Wallenstein *et al*. (2009) suggested that decomposition in arctic tundra soils may be limited to low extracellular microbial enzyme activities, which are limited by nitrogen availability. They found that enzyme activity declined with N availability over the growing season in tussock tundra. However, in shrub tundra, enzyme activity remained constant or increased through the growing season, which may be linked with greater N availability in shrub than tussock tundra through the growing season (Weintraub and Schimel 2005). These results support my observed increase in root turnover in soils with nutrient addition compared to the control. The plots with nutrient addition are becoming an intermediary between control tussock tundra and shrub tundra in terms of vegetation cover (e.g. Chapin *et al.* 1995; Shaver *et al.* 2001), but also in terms of microbial biomass; for example, Clemmensen *et al.* (2006) observed an increase in fungal biomass with nutrient addition in tussock tundra, and others have observed that shrub tundra fungal biomass > tussock tundra biomass under nutrient addition > control tussock tundra biomass (Gough *et al.* in preparation; Moore *et al.* in preparation).

Increases in root production and turnover rates may be due to increased root herbivory, which affect plant biomass production as well as resource allocation, and may affect fine root growth and the production of root exudates (Bardgett and Wardle 2003). In the same plots sampled for the present study, Gough *et al*. (in preparation) found no phytophagous nematodes in the mineral soil of control plots, but did observe a slight increase in nematode biomass (5.179×10^{-7} mg/g soil) in mineral soils with nutrient addition since 1989, but not in organic soil. In grassland systems, root biomass production is enhanced through infection by root-feeding nematodes, which may also result in increased nutrient inputs in the soil (Bardgett *et al*. 1999), resulting in increased microbial activity and organic matter turnover (Yeates *et al*. 1998). Root herbivory, combined with active microbial enzyme production through the growing season, may result in increased light fraction production with nutrient addition. Light fraction carbon is correlated with microbial activity (Alvarez and Alvarez 2000) and aggregate formation and stabilization (Miller and Jastrow 1990).

Observations of increases in light fraction and iPOM carbon and nitrogen alongside decreases in root biomass, when coupled with the observations of Wallenstein *et al*. (2009) and Moore *et al*. (unpublished), suggest that a linkage between SOM dynamics, microbial activity, and root herbivory may exist in the Arctic. Aggregate formation is a microbially-mediated process. An increase in root turnover was observed, along with nutrient addition coupled with increases in light fraction and iPOM carbon as well as light fraction nitrogen. Light fraction quality (lower C:N) is improving (Fig. 3.11), but it is still >20:1, which means nitrogen would still be immobilized within the microbial community. This limitation on microbial activity may inhibit further aggregate formation and stabilization. Even though the light fraction carbon and nitrogen increased with nutrient addition, this fraction should still be considered unstable compared to mineral-associated SOM and iPOM (Ashagrie *et al*. 2007), and may have been the fraction that contributed to a seasonal loss of carbon that was observed in plots with nutrient addition in 2006 (See Chapter 1). In future studies of SOM dynamics in the Arctic, it would be useful to investigate temperature sensitivity of POM fractions. It is possible that further decomposition may be limited not only by nitrogen, but temperature as well, given that when SOM is decomposed, its temperature sensitivity has been shown to increase with increased humification/recalcitrance. (Conant *et al*. 2008).

Conclusions

The re-allocation of SOM from physically protected aggregates to light fraction with nutrient addition may result in shifts in SOM stability in these soils. More nitrogen was allocated to the light fraction in soils with nutrient addition than in control soils, which increased the decomposability of light fraction SOM. At the same time, the amount of iPOM carbon increased with nutrient addition. These two SOM fractions have different levels of sensitivity to decomposition. Light fraction decomposition is mainly influenced by soil temperature, soil moisture, and residue quality and input, whereas iPOM decomposition is primarily affected by aggregate turnover dynamics (Six *et al*. 1999a).

Nutrient addition results in changes in SOM dynamics during the growing season. The observed increases in the proportion of soil carbon as light fraction and iPOM with nutrient addition indicate a shift towards an increase in POM fractions that tend to be labile, potentially mineralizable sources of organic matter.

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CHAPTER FOUR

INTEGRATION OF RESULTS INTO A CONCEPTUAL MODEL OF SOIL ORGANIC MATTER STABILIZATION WITHIN AGGREGATES

Section 4.1. Introduction

The objective of the research conducted for this dissertation has been to determine how soil organic matter (SOM) is distributed within the active mineral layer of tussock tundra soil, and to determine how SOM is affected by long-term nutrient addition. In the previous two chapters, results on SOM and aggregate fractionations were presented. In this chapter, the key findings of Chapter 2 and Chapter 3 are briefly outlined. Relationships between the chapters are then discussed, along with how this work relates to the research of others, and suggest how future research could proceed.

Section 4.1 Subsection 1: Chapter 2 summary

Chapter 2 focused on soil aggregate distribution along with carbon and nitrogen association with aggregates. The key findings of Chapter 2 were:

- Aggregate size distribution did not differ with nutrient addition in early June, but did by mid-June, when control soils had more macroaggregates and fewer microaggregates than soils with nutrient addition. By late June and August, however, there were no longer differences in aggregate size distribution.

- By late June, the whole soil C:N was lower in soils with nutrient addition since 1989 than other soils.
- The ratio of free:occluded microaggregates was >1 for soils with nutrient addition, except in early June. The ratio was <1 for control soils in June, but by August was >1, which means there were more free microaggregates than occluded ones.
- As the ratio of free:occluded microaggregates rose over the growing season, so did the nitrogen content of free:occluded microaggregates coupled with a lower C:N.

The results from Chapter 2 provide evidence that aggregate formation and disruption occur during the course of the growing season. Specifically, the ratio of free:occluded microaggregates rose over the growing season, which means that microaggregates held within macroaggregates may have been released upon macroaggregate disruption. Occluded microaggregates tend to possess higher carbon and nitrogen contents than free microaggregates due to increased physical protection within the macroaggregate. Because of this, the ratio of free:occluded microaggregate nitrogen content rose over the growing season, possibly due to nitrogen-rich, formerly occluded microaggregates entering the free microaggregate pool.

Within the aggregate fractions, a lower C:N was measured in aggregates from soils with nutrient addition than control soils. Effects of nutrient addition on aggregate size distribution were observed in mid-June, but there were no differences at the beginning and end of the growing season. At the same time, nutrient addition has affected the plant and microbial community, so shifts in aggregate structure may impact the occlusion of organic matter as it enters the SOM pool. In order to examine this process further, the distribution of particulate organic matter as either light fraction or intra-aggregate particulate organic matter (iPOM) was measured, and presented in Chapter 3.

Section 4.1 Subsection 2: Chapter 3 summary

Chapter 3 focused on the location of particulate organic matter (POM) as either occluded within aggregate structure as iPOM or free, non-occluded light fraction organic matter (LF). The key findings of Chapter 3 were:

- In mid-June, the percentage of small macroaggregate C as iPOM was greater in the soils with nutrient addition since 1989 than the control. In late June, the percentage of microaggregate C as iPOM was greater in soils with nutrient addition since 1989 than soils with nutrient addition since 1996.
- The percent of whole soil carbon as both small macroaggregate and microaggregate LF increased over the growing season in soils with nutrient addition since 1989.
- The percent of small macroaggregate N as LF-N increased over the growing season for both nutrient addition treatments, though significantly only for soils with nutrient addition since 1989.
- The C:N declined in light fraction in soils with nutrient addition since 1989.
- Whole soil root biomass increased in June, then dropped by August. Root biomass was greater in soils with nutrient addition than the control.

The results from Chapter 3 provide evidence that both the rate of organic matter input and decomposition both increase with nutrient addition. The proportion of whole soil carbon associated with the macroaggregate light fraction increased over the growing season, as did the microaggregate iPOM fraction. These two results may provide evidence that organic matter processing is increasing, and that it is becoming occluded within aggregates. The coarse light fraction associated with macroaggregates may become occluded within newly formed macroaggregates, and may have been sites of microaggregate formation within the macroaggregate (Angers *et al*. 1997; Oades 1984).

Live root biomass was observed to increase through late June in soils with nutrient addition as evidence of an increase in the rate of plant growth. By the end of the season, root biomass declined. If the rate of plant growth and subsequent organic matter inputs increased at the same rate as organic matter decomposition, then the C:N of light fraction particulate organic matter should remain relatively stable, as it did in control plots. However, the C:N declined in the light fraction of soils with nutrient addition since 1989. This means that more carbon is being respired than is entering the system because it is likely that nitrogen is being conserved within the system.

Taken together, the results from Chapter 2 and Chapter 3 do not provide evidence of carbon loss from the system as found by Mack *et al*. (2004), but instead suggest a reallocation of soil organic matter within the soil matrix. Terrestrial carbon storage depends on a balance between rates of input due to net primary productivity and decomposition (Arneth *et al*. 2010; Jahn *et al*. 2010). It is likely that the rate of both processes has increased with nutrient addition. The effects of changes in soil organic matter allocation and processing rates in Arctic systems are not yet clear, and in the next section postulations are made on possible interactions with other components of terrestrial systems, including the belowground foodweb and microbial activity.

Section 4.2. Soil Organic Matter, microbes, food web, and nutrient addition

In the following sub-sections, brief descriptions of previous findings on relationships between nutrient addition and food webs, microbes, and aggregates/POM are provided as pieces of evidence of both nutrient addition and the interactivity of components within arctic terrestrial systems. Discussion as to how these relationships form linkages to soil organic matter (SOM) in arctic soils is provided in Section 3.

Section 4.2 Subsection 1: Linkages between nutrient addition and foodweb dynamics

Gough *et al*. (in preparation) have observed an increase in phytophagous nematodes with nutrient addition in the mineral layer of tussock tundra soils. An increase in phytophagous nematodes may lead to an increase in root herbivory. In June, an increase in live root biomass was observed in the mineral soil with nutrient addition, but by August, this declined. This loss of root biomass may be due to the addition of a new trophic level at the mineral soil depth (herbivores). If herbivores are colonizing soils under nutrient addition in my study, a range of effects may result, including increased organic matter processing. In other systems, the addition of a new trophic level altered nutrient cycling dynamics (Carpenter *et al*. 1985).

Section 4.2 Subsection 2: Linkages between nutrient addition and microbial activity

Tussock tundra soils are N-limited (Hobbie and Chapin 1998), particularly at the end of the growing season. The largest flush of nutrients occurs at spring thaw then declines throughout the growing season. Shrub tundra, on the other hand, is not N-limited at the end of the growing season (Weintraub and Schimel 2005). Long-term nutrient addition experiments in tussock tundra may have helped alleviate this N-limitation.

Wallenstein *et al*. (2009) suggested that microbial enzyme activity is N-limited in tussock tundra, which would result in a decline in microbial activity by the end of the growing season. They did not observe this decline in microbial enzyme activity in shrub tundra. If nutrient addition has alleviated nutrient limitations in tussock tundra plots, then continued microbial activity throughout the growing season may lead to increased decomposition. An increase in decomposition would result in increased light fraction production, which is what was observed in soils with nutrient addition (Chapter 3). Nutrient availability tends to increases as decomposition rates increase, but only for a finite period of time. Eventually as soil organic pools are decomposed, leaving increasingly humified material, mineral nitrogen availability will decrease (Luo 2007).

<u>Section 4.2 Subsection 3: Linkages between nutrient addition and aggregate/particulate organic matter dynamics</u>

Nutrient addition in tussock tundra soils has resulted in an increased allocation of SOM to particulate organic matter. The amount of SOM as both light fraction and intra-aggregate particulate organic matter (iPOM) has increased, and silt+clay-associated organic matter has decreased in small macroaggregates and microaggregates. Total soil carbon and nitrogen content did not change, but their distribution did.

Over the course of the growing season, the C:N of light fraction and aggregates declined, which may indicate that SOM decomposition is occurring, which reduces carbon content through respiration while largely conserving nitrogen in increasingly complex SOM structures and microbial biomass. In addition, the C:N of free microaggregates became more similar to occluded microaggregates at the end of the growing season in control soils (Chapter 2). These results may suggest that in control soils, there is macroaggregate turnover at the end of the growing season, which may result in a release of previously occluded microaggregates to the free microaggregate fraction. If new aggregates are forming at the beginning of the growing season, then macroaggregate turnover mid-late season would be in agreement with previous studies that demonstrated that macroaggregate mean residence time could range from 30-95 days (Plante *et al*. 2002; De Gryze *et al*. 2006).

Section 4.3. Conceptual model of relationships between aggregates, POM, and biota with nutrient addition

In the previous section, the relationships between nutrient addition in tussock tundra and foodweb, microbial, and aggregate/POM dynamics were discussed. The results from this dissertation, taken with the results of Gough *et al*. (in preparation), and Wallenstein *et al*. (2009), may be used to form a conceptual model of the relationships between aggregates/POM and the biotic community. Nutrient addition in tussock tundra results in a shift from old, recalcitrant organic matter to a relatively young pool (Nowinski *et al*. 2008) of particulate organic matter (POM). A conceptual model is presented in Figure 1. The mechanism of this shift is envisioned as follows:

Nutrient addition leads to increased root infiltration into the active mineral layer. This increase in plant growth occurs early in the growing season (June). This increase in root growth at lower depths, combined with increased nitrogen, enables the belowground community to remain active throughout the growing season and to exist below the organic horizon into the mineral soil layer (Moore *et al*. in preparation). The active belowground community affects soil aggregate and POM dynamics. Phytophagous nematodes, which Gough *et al*. (in preparation) found in tussock tundra with nutrient addition, but not in unfertilized soil, may consume live roots, which would lead to the observed decrease in live root biomass. Root herbivory results in root fragmentation and exudate production. A large amount of root-based organic matter is lost from plants and

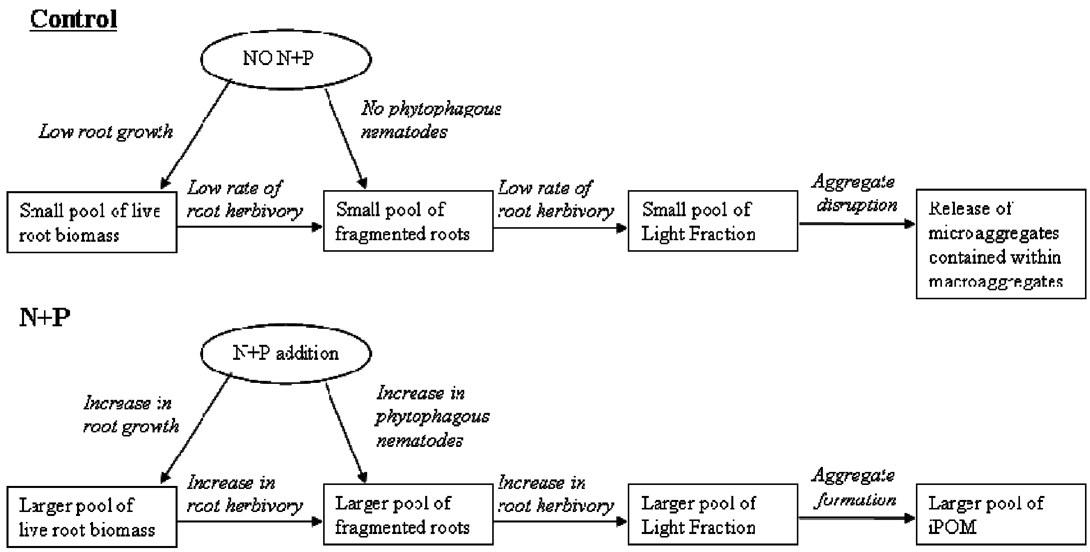

Figure 4.1. Conceptual model of relationships between plants, soil biota, and soil organic matter with nutrient addition.

is consumed by microbes (Merckx *et al*. 1985). Soil organic matter in the arctic may be considered to be 'suspended' rather than stabilized due to low rates of humification processes (Davidson and Janssens 2006). In other systems that contain more processed organic matter, the addition of fresh substrate results in accelerated SOM decomposition of both old SOM and new residues (Hallam and Bartholomew 1953), which is termed the "priming effect" (Parnas 1976). Shifts in plant community structure and the amounts of root inputs can alter decomposition rates (Dormaar 1990).

Under control conditions in tussock tundra, microbial enzyme activity is N-limited by the end of the growing season, but is not in shrub tundra, which does not have a period of N-limitation during the growing season (Wallenstein *et al*. 2009). Long-term nutrient addition in tussock tundra may lead to a hybrid system between tussock and shrub tundra in which nitrogen is not limiting at the end of the growing season, resulting in continued microbial enzyme activity. The result of continued microbial enzyme activity and root herbivory in soils with nutrient addition is an increase in light fraction organic matter. The light fraction is also decomposed, which results in a lowering of its C:N ratio. As the light fraction is decomposed, some of it becomes occluded within aggregate structures, resulting in an increase in intra-particulate organic matter (iPOM).

Control tussock tundra soils are not as active in the mineral layer as soils with nutrient addition ones due to nutrient limitations and a lack of root infiltration, which results in less light fraction production and subsequent integration into aggregates as iPOM. Because aggregate formation may occur at a lower rate in control soils, macroaggregates may begin to break down by the end of the growing season, releasing microaggregates contained within them. These microaggregates, which have a lower C:N than previously free microaggregates, lower the collective free microaggregate C:N ratio upon their entry into this fraction. Faster SOM turnover with nutrient addition has been previously noted by other researchers. Nowinski *et al*. (2008) determined that long term nutrient addition in tussock tundra results in faster carbon turnover than in control mineral soils.

Section 4.4. Long-term effects/Unanswered Questions/ Future Work

Terrestrial carbon storage in arctic systems is predicted to decline with increasing temperature (Schuur *et al*. 2009; White *et al*. 2004 Oechel *et al*. 1993). It is important to understand how feedbacks on soil carbon loss may affect long-term carbon storage dynamics. For instance Waelbroeck *et al*. (1997) have predicted that carbon dioxide efflux would increase, followed by a longer period of carbon accumulation in response to partial permafrost thawing and increases in both decomposition and nutrient availability. Stieglitz *et al*. (2006) modeled the effects of an increased active layer in arctic soils and estimated that carbon at lower depths would decompose, resulting in a lower soil carbon residence time, but raised the question that the extent to which this carbon loss will occur is unknown.

The preliminary results from 2006 (Chapter 1) suggested that aggregate structure and growing season carbon accumulation were related. As the aggregate mean weight-diameter (MWD) increased, so did carbon accumulation. In the control soils, the MWD increased more than in soils with nutrient addition. At the same time, control soils accumulated carbon whereas soils with nutrient addition remain relatively neutral in terms of carbon accumulation. However, in 2007, the same increase in MWD and carbon accumulation in control soils was not observed. These results suggest that aggregate formation and SOM stabilization are dynamic within arctic systems, and the controls on these processes are not yet as fully understood and predictable as they are in temperate systems (Six *et al*. 2004).

Long-term experiments are necessary in order to elucidate mechanisms that affect SOM dynamics in arctic tundra. On a large scale, carbon balance measurements at the ecosystem scale such as the work of Mack *et al*. (2004) provide a snapshot of long-term nutrient addition affects and subsequent shifts in vegetation and microbial community composition and dynamics. The work within this dissertation illustrates that multiple sampling efforts over a growing season provide an added dimension of arctic terrestrial carbon research, which has been shown to be dynamic, both intra- and inter-seasonally. Aggregation and SOM turnover may undergo multi-year cycles. For instance, the high quality (low C:N) free microaggregates left at the end of the growing season in control soils may result in the formation of highly stable macroaggregates the following year, resulting in carbon accumulation. Conversely, if increases in nitrogen availability drive decomposition and does not result in physical protection of SOM through aggregate formation, then carbon loss will occur.

In the future, work that fully integrates SOM dynamics with the plant community, belowground community, including microbial enzyme activity will aid in our understanding of carbon storage in tussock tundra and the mechanisms of its stabilization. In particular, the role of amino acids as a source of nitrogen for both plants and microbes is of interest in a nitrogen-limited system (Chapin *et al*. 1993; Kielland 1994; Schimel and Bennett 2004), along with the effect of increases in temperature on decomposition (Conant *et al*. 2008; Kirschbaum 2006). As rising temperatures in the arctic remove constraints, including nutrient availability, on both decomposition and NPP (e.g. Shaver *et al.* 1992), understanding how aggregate formation and SOM stabilization moderate the interaction between these processes will aid in our ability to predict carbon gain/loss from arctic systems.

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APPENDIX A

AGGREGATE MEAN WEIGHT-DIAMETER AND WHOLE SOIL CARBON CONTENT FROM 2006.

Table A1: Aggregate MWD and whole soil carbon content from 2006.

Table A1. Aggregate mean weight-diatmeter and whole soil carbon content.

Treatment	Date	Block	MWD (μm)	Whole soil Carbon content (% C)
Control	June	1	1198.48	3.90
		2	840.10	3.44
		3	801.05	3.26
		4	931.90	3.66
	July	1	2434.74	5.37
		2	1244.65	6.26
		3	1725.76	5.69
		4	965.80	3.78
N+P since 1996	June	1	866.33	4.57
		2	2619.32	3.94
		3	713.80	2.79
		4	851.46	4.33
	July	1	1244.02	4.72
		2	939.65	2.98
		3	1685.92	3.95
		4	1301.68	3.58
N+P since 1989	June	1	935.43	4.73
		2	no data	no data
		3	542.19	2.46
		4	892.80	6.60
	July	1	1702.54	4.89
		2	795.68	3.80
		3	1163.90	3.39
		4	1075.75	3.59

APPENDIX B

AGGREGATE SIZE DISTRIBUTION AND SMALL MACROAGGREGATE SUB-FRACTION SIZE DISTRIBUTION

Table B1: Aggregate size distribution from Control soils.

Table B2: Aggregate size distribution from soils with nutrient addition since 1996.

Table B3: Aggregate size distribution from soils with nutrient addition since 1989.

Table B4: Small macroaggregate sub-fraction size distribution from Control soils.

Table B5: Small macroaggregate sub-fraction size distribution from soils with nutrient addition since 1996.

Table B6: Small macroaggregate sub-fraction size distribution from soils with nutrient addition since 1989.

Table B1. Aggregate fraction size distribution (Control soils)

			Aggregate size distribution (% of whole soil)				
Treatment	**Date**	**Block**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**Mean Weight-Diameter (µm)**
Control	Early June	1	11.35	59.55	17.58	11.52	1267.31
		2	5.33	51.64	25.77	17.26	890.94
		3	8.96	57.34	19.87	13.83	1126.95
		4	7.61	55.65	22.99	13.74	1045.04
	Mid June	1	5.97	58.22	20.98	14.82	989.36
		2	5.15	57.08	24.32	13.45	940.04
		3	5.94	60.51	21.22	12.33	1013.27
		4	8.84	59.67	19.96	11.53	1146.49
	Late June	1	32.17*	38.78*	16.55*	12.50*	2073.10*
		2	1.83	59.10	24.68	14.39	797.52
		3	7.00	64.07	17.57	11.36	1100.48
		4	4.73	50.78	29.14	15.36	855.77
	August	1	12.84	54.85	18.58	13.72	1290.99
		2	0.72	43.35	35.02	20.92	582.13
		3	16.70	36.40	29.93	16.97	1294.34
		4	10.04	52.47	22.94	14.55	1130.76

*Only organic soil: not used for analysis.

Table B2. Aggregate fraction size distribution (N+P since 1996)

			Aggregate size distribution (% of whole soil)				
Treatment	**Date**	**Block**	**>2000 μm**	**250-2000 μm**	**53-250μm**	**<53μm**	**Mean Weight-Diameter (μm)**
N+P since 1996	Early June	1	13.54	53.73	19.22	13.51	1314.01
		2	2.26	59.22	24.24	14.28	819.77
		3	10.19	55.80	21.37	12.64	1172.90
		4	12.40	66.31	13.66	7.63	1388.78
	Mid June	1	3.07	49.00	29.93	18.00	754.78
		2	3.75	56.84	25.11	14.30	868.78
		3	4.39	39.68	33.51	22.42	722.72
		4	13.77	39.94	26.91	19.38	1337.73
	Late June	1	1.92	54.15	25.69	18.24	748.81
		2	3.79	58.59	22.98	14.64	887.11
		3	1.08	33.81	39.84	25.27	501.55
		4	1.14	54.26	28.73	15.87	715.26
	August	1	6.17	60.93	20.31	12.58	1028.00
		2	2.36	50.18	31.09	16.36	734.15
		3	22.96	53.33	14.88	8.83	1773.04
		4	2.18	38.80	31.08	27.93	600.16

Table B3. Aggregate fraction size distribution (N+P since 1989)

			Aggregate size distribution (% of whole soil)				
Treatment	**Date**	**Block**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**Mean Weight-Diameter (µm)**
N+P since 1989	Early June	1	1.68	48.88	31.72	17.72	686.64
		2	2.25	63.02	22.06	12.66	858.47
		3	1.09	45.03	33.15	20.74	616.57
		4	8.76	64.64	16.07	10.53	1192.14
	Mid June	1	1.71	59.29	25.73	13.27	794.92
		2	2.73	52.39	28.78	16.09	773.87
		3	0.46	44.52	33.57	21.45	581.66
		4	4.13	62.23	21.12	12.52	941.70
	Late June	1	1.50	43.66	32.93	21.92	621.69
		2	14.60	43.18	26.04	16.19	1259.28
		3	0.18	43.11	34.66	22.06	552.26
		4	5.80	55.27	22.48	16.46	950.25
	August	1	1.96	50.50	29.91	17.63	715.91
		2	3.26	52.92	26.29	17.53	802.59
		3	14.65	50.26	22.94	12.16	1335.90
		4	6.39	43.42	30.14	20.05	858.80

Table B4. Small macroaggregate sub-fraction distribution (Control soils)

			Small macroaggregate sub-fraction distribution (percent of small macroaggregate)			Whole soil content of small macroaggregate sub-fractions (mg fraction/g whole soil)		
			Aggregate sub-fraction size			Aggregate sub-fraction size		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**
Control	Early June	1	18.59	49.34	32.07	110.82	293.47	191.23
		2	18.21	45.51	36.28	93.61	235.27	187.55
		3	18.97	47.77	33.26	107.54	272.79	193.05
		4	19.74	48.73	31.53	108.09	272.69	175.74
	Mid June	1	22.28	48.24	29.47	127.63	281.51	173.05
		2	14.51	55.79	29.70	81.72	318.46	170.63
		3	17.95	52.65	29.40	108.13	318.61	178.32
		4	18.64	49.41	31.95	111.26	294.43	191.05
	Late June	1	organic soil: Not analyzed			organic soil: Not analyzed		
		2	14.27	58.78	26.95	84.34	347.38	159.24
		3	13.95	57.85	28.20	89.87	370.80	180.00
		4	19.46	55.15	25.39	98.75	280.28	128.78
	August	1	19.37	47.06	33.58	105.77	258.25	184.52
		2	15.84	51.56	32.59	68.68	223.53	141.29
		3	21.28	43.85	30.94	80.72	159.69	116.12
		4	17.48	52.34	30.18	91.33	275.15	158.25

Table B5. Small macroaggregate sub-fraction distribution (N+P since 1996)

			Small macroaggregate sub-fraction distribution (percent of small macroaggregate)			Whole soil content of small macroaggregate sub-fractions (mg fraction/g whole soil)		
			Aggregate sub-fraction size			Aggregate sub-fraction size		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1996	Early June	1	22.69	45.35	31.96	124.55	241.59	171.20
		2	23.75	47.24	29.01	131.29	287.45	173.44
		3	18.37	53.77	27.87	102.54	300.05	155.45
		4	18.22	55.22	26.57	120.79	366.14	176.15
	Mid June	1	19.63	54.08	26.29	93.58	264.67	131.77
		2	23.49	50.17	26.34	129.52	287.82	151.09
		3	29.29	45.83	24.88	101.30	195.21	100.31
		4	37.57	43.21	24.51	108.36	185.11	100.28
	Late June	1	18.02	51.24	30.87	97.11	277.54	167.12
		2	19.98	50.86	28.37	128.80	297.60	167.09
		3	26.75	42.60	23.48	103.74	145.71	81.25
		4	24.83	54.14	22.64	120.68	294.08	123.46
	August	1	14.42	53.49	32.09	87.37	325.25	196.73
		2	29.86	49.25	21.38	157.41	246.79	107.48
		3	14.98	50.05	34.96	79.92	266.94	186.48
		4	24.78	35.47	20.41	135.51	157.00	90.59

Table B6. Small macroaggregate sub-fraction distribution (N+P since 1989)

			Small macroaggregate sub-fraction distribution (percent of small macroaggregate)			Whole soil content of small macroaggregate sub-fractions (mg fraction/g whole soil)		
			Aggregate sub-fraction size			Aggregate sub-fraction size		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1989	Early June	1	21.42	51.31	27.27	104.34	250.96	133.54
		2	17.41	49.89	32.69	108.31	315.58	206.28
		3	19.07	48.45	28.99	106.47	221.17	130.35
		4	15.12	55.13	29.75	97.92	356.48	191.97
	Mid June	1	18.34	52.77	28.89	106.27	312.23	174.37
		2	18.68	49.95	27.55	111.08	268.21	143.14
		3	24.79	45.51	25.82	138.90	205.23	116.90
		4	15.86	56.15	27.99	98.76	349.91	173.62
	Late June	1	24.66	47.73	27.61	105.49	209.02	122.09
		2	27.16	45.20	27.65	117.26	195.15	119.37
		3	24.96	48.97	26.07	103.39	213.48	114.19
		4	21.37	49.31	29.32	118.30	272.34	162.04
	August	1	19.61	54.99	27.56	92.75	277.87	139.17
		2	22.43	44.10	32.51	115.53	237.63	173.77
		3	16.45	54.10	28.25	92.24	273.66	141.77
		4	29.79	47.73	28.14	123.81	207.30	122.43

APPENDIX C

AGGREGATE CARBON CONTENT, NITROGEN CONTENT, AND C:N

Table C1. Aggregate carbon content (Control Soils)

			Aggregate Carbon concentration (mg C/g fraction)				Whole Soil Aggregate Carbon (mg C in fraction/g whole soil)			
			Aggregate size fraction				Aggregate size fraction			
Treatment	**Date**	**Block**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**
Control	Early June	1	55.48	51.24	45.74	63.07	6.21	30.59	8.06	7.26
		2	57.85	40.81	31.29	38.36	3.71	21.21	7.90	6.56
		3	40.15	36.48	30.22	45.48	3.97	21.13	5.56	5.99
		4	44.45	37.29	30.47	40.82	3.45	20.59	7.08	5.69
	Mid June	1	67.69	60.99	50.75	63.60	4.26	34.99	10.71	9.50
		2	62.79	54.08	43.72	43.33	3.02	31.26	10.73	5.92
		3	39.23	34.31	29.84	42.07	2.46	20.68	6.27	5.13
		4	40.47	40.00	35.50	45.92	3.82	23.85	7.01	5.24
	Late June	1	organic soil: Not analyzed for C and N				organic soil: Not analyzed for C and N			
		2	44.27	35.22	27.62	35.91	0.81	20.81	6.82	5.17
		3	43.07	41.12	34.40	45.67	3.14	26.07	6.00	5.18
		4	43.94	35.26	27.38	37.72	2.08	17.84	8.01	5.81
	August	1	51.99	49.14	40.19	62.74	6.60	26.84	7.45	8.72
		2	57.78	42.94	30.95	36.23	0.41	18.61	10.84	7.58
		3	38.04	32.19	29.67	46.38	10.28	11.83	8.37	7.60
		4	55.05	39.04	26.98	37.37	5.58	20.80	6.08	5.40

Table C2. Aggregate carbon content (N+P since 1996)

			Aggregate Carbon concentration (mg C/g fraction)				Whole Soil Aggregate Carbon			
			Aggregate size fraction				Aggregate size fraction			
Treatment	**Date**	**Block**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1996	Early June	1	62.03	52.30	43.33	50.50	9.00	27.49	8.38	6.83
		2	40.20	38.99	35.46	49.00	0.95	23.64	8.19	6.81
		3	33.83	29.16	23.31	35.31	3.60	16.25	4.98	4.41
		4	74.38	61.61	47.02	48.46	9.22	40.85	6.42	3.70
	Mid June	1	57.56	44.70	38.14	48.60	1.83	21.65	11.48	8.81
		2	50.40	46.51	41.54	53.89	1.97	26.91	10.16	7.40
		3	43.44	26.18	21.68	32.97	2.04	10.56	7.17	7.34
		4	47.27	39.63	29.42	33.64	11.90	17.59	7.17	5.50
	Late June	1	41.15	39.11	29.77	39.82	0.92	21.19	7.65	7.26
		2	51.99	49.31	45.14	61.08	3.24	29.05	10.33	8.84
		3	16.91	24.94	22.65	35.01	0.12	8.67	8.92	8.75
		4	37.97	34.56	32.42	42.03	0.83	18.97	8.93	6.63
	August	1	60.69	53.86	44.22	54.43	3.49	32.45	9.26	6.94
		2	44.85	21.67	23.19	37.18	0.46	10.89	7.21	6.09
		3	28.50	28.13	24.46	36.91	6.54	15.00	3.64	3.26
		4	42.58	23.35	18.80	28.00	1.85	11.26	5.47	7.50

Table C3. Aggregate carbon content (N+P since 1989)

			Aggregate Carbon concentration (mg C/g fraction) — Aggregate size fraction				Whole Soil Aggregate Carbon — Aggregate size fraction			
Treatment	**Date**	**Block**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1989	Early June	1	41.76	44.71	40.04	49.79	0.76	21.79	12.81	8.83
		2	40.50	33.71	29.70	38.80	0.99	21.62	6.31	4.77
		3	33.91	36.56	31.99	42.32	0.93	16.19	10.94	8.92
		4	66.34	52.36	40.16	50.10	5.68	33.83	6.47	5.29
	Mid June	1	51.05	45.51	41.07	49.68	0.84	26.81	10.69	6.62
		2	46.48	29.54	24.72	32.32	1.74	16.20	6.84	5.03
		3	63.76	35.83	31.75	42.50	0.50	16.76	10.22	9.06
		4	50.64	43.81	35.34	44.23	2.09	27.33	7.45	5.50
	Late June	1	62.09	40.43	32.06	41.80	0.93	17.82	10.45	9.13
		2	130.30*	51.99	31.20	39.48	19.02*	22.45	8.12	6.39
		3	no sample†	33.57	31.94	43.94	no sample†	14.43	11.29	9.69
		4	37.72	35.26	26.06	38.08	2.39	19.44	5.80	6.25
	August	1	41.23	49.37	39.82	47.16	0.36	24.88	11.61	8.42
		2	44.53	28.56	23.54	31.58	2.59	16.00	5.82	5.30
		3	52.09	47.03	35.05	41.95	4.77	22.44	7.97	5.10
		4	49.48	58.29	48.17	54.97	0.44	25.35	14.49	11.07

*No aggregates in sample - only large organic matter. Not used in analysis.

†Not enough sample for carbon analysis.

Table C4. Aggregate nitrogen content (Control Soils)

			Aggregate Nitrogen concentration (mg N/g fraction)				Whole Soil Aggregate Nitrogen (mg N in fraction/g whole soil)			
			Aggregate size fraction				Aggregate size fraction			
Treatment	**Date**	**Block**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**
Control	Early June	1	2.14	2.10	1.85	2.89	0.24	1.26	0.33	0.33
		2	2.22	1.81	1.42	2.01	0.14	0.94	0.36	0.34
		3	1.72	1.41	1.21	1.99	0.16	0.81	0.23	0.27
		4	1.85	1.47	1.15	1.84	0.14	0.82	0.26	0.25
	Mid June	1	3.10	2.73	2.24	3.12	0.20	1.56	0.47	0.47
		2	3.03	2.67	2.09	2.38	0.14	1.56	0.50	0.32
		3	1.75	1.44	1.33	2.05	0.11	0.86	0.28	0.25
		4	1.58	1.62	1.41	2.17	0.14	0.97	0.28	0.25
	Late June	1	organic soil: Not analyzed for C and N				organic soil: Not analyzed for C and N			
		2	1.92	1.65	1.23	1.86	0.04	0.98	0.30	0.27
		3	1.59	1.54	1.21	1.92	0.11	0.98	0.21	0.22
		4	1.68	1.30	0.95	1.84	0.08	0.66	0.28	0.28
	August	1	2.11	1.83	1.62	2.93	0.27	0.99	0.31	0.41
		2	2.79	2.36	1.71	2.40	0.02	1.02	0.60	0.50
		3	1.62	1.59	1.35	2.23	0.43	0.62	0.31	0.25
		4	2.36	1.86	1.32	2.02	0.25	0.99	0.29	0.29

Table C5. Aggregate nitrogen content (N+P since 1996)

			Aggregate Nitrogen concentration (mg N/g fraction)				Whole Soil Aggregate Nitrogen			
			Aggregate size fraction				Aggregate size fraction			
Treatment	**Date**	**Block**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1996	Early June	1	2.63	2.33	1.90	2.61	0.38	1.22	0.37	0.35
		2	1.82	1.62	1.46	2.37	0.04	0.98	0.34	0.33
		3	2.08	1.19	0.89	1.69	0.21	0.66	0.19	0.21
		4	3.83	3.23	2.51	3.06	0.48	2.14	0.34	0.23
	Mid June	1	2.65	1.91	1.82	2.49	0.08	0.93	0.54	0.45
		2	2.16	1.82	1.57	2.53	0.08	1.05	0.39	0.35
		3	1.83	1.17	0.96	1.64	0.08	0.47	0.31	0.36
		4	2.50	1.50	1.17	1.71	0.62	0.50	0.30	0.34
	Late June	1	1.91	1.59	1.12	1.98	0.04	0.86	0.29	0.36
		2	2.13	2.01	1.65	2.83	0.13	1.30	0.30	0.32
		3	0.84	0.98	1.21	1.70	0.01	0.38	0.45	0.40
		4	1.79	1.27	0.98	1.51	0.04	0.62	0.32	0.24
	August	1	2.76	2.41	1.89	2.90	0.16	1.45	0.40	0.37
		2	1.77	1.13	1.29	2.00	0.02	0.59	0.38	0.34
		3	1.18	1.25	1.20	1.86	0.27	0.67	0.18	0.16
		4	2.07	2.00	1.68	2.09	0.09	1.09	0.43	0.33

Table C6. Aggregate nitrogen content (N+P since 1989)

			Aggregate Nitrogen concentration (mg N/g fraction)				Whole Soil Aggregate Nitrogen			
			Aggregate size fraction				Aggregate size fraction			
Treatment	**Date**	**Block**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1989	Early June	1	1.68	1.84	1.57	2.26	0.03	0.90	0.50	0.40
		2	2.31	1.71	1.45	2.26	0.06	1.09	0.31	0.28
		3	1.56	1.27	1.03	1.79	0.04	0.71	0.26	0.29
		4	3.16	2.33	1.74	2.60	0.28	1.50	0.28	0.27
	Mid June	1	2.21	1.81	1.58	2.23	0.04	1.07	0.41	0.30
		2	2.55	1.85	1.42	2.18	0.10	1.10	0.35	0.26
		3	3.00	2.24	1.89	2.60	0.02	1.26	0.49	0.45
		4	2.28	2.02	1.67	2.39	0.10	1.26	0.35	0.30
	Late June	1	2.62	1.91	1.51	2.46	0.04	0.84	0.49	0.54
		2	5.67*	2.72	1.69	2.31	0.83*	1.17	0.44	0.37
		3	no sample†	1.26	1.72	2.12	no sample†	0.55	0.64	0.47
		4	1.99	1.66	1.22	2.17	0.12	0.92	0.27	0.36
	August	1	1.63	1.50	1.24	2.05	0.01	0.71	0.43	0.35
		2	2.19	1.87	1.46	2.14	0.13	1.11	0.32	0.32
		3	2.70	1.87	1.52	2.28	0.24	1.05	0.36	0.25
		4	2.82	2.93	2.38	3.38	0.02	1.22	0.80	0.81

*No aggregates in sample - only large organic matter. Not used in analysis.

†Not enough sample for carbon analysis.

Table C7. Aggregate carbon:nitrogen ratio (Control Soils)

			Aggregate fraction Carbon:Nitrogen			
			Aggregate size fraction			
Treatment	**Date**	**Block**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**
Control	Early June	1	25.91	24.43	24.78	21.84
		2	26.01	22.61	22.00	19.06
		3	23.37	25.86	25.03	22.84
		4	24.08	25.35	26.45	22.13
	Mid June	1	21.84	22.34	22.69	20.37
		2	20.70	20.26	20.95	18.18
		3	22.38	23.91	22.39	20.50
		4	25.54	24.66	25.09	21.18
	Late June	1	organic soil: Not analyzed for C and N			
		2	23.01	21.31	22.53	19.33
		3	27.16	26.64	28.34	23.84
		4	26.21	27.18	28.68	20.53
	August	1	24.62	26.91	24.80	21.43
		2	20.68	18.20	18.07	15.13
		3	23.52	20.31	21.97	20.84
		4	23.35	21.04	20.44	18.51

Table C8. Aggregate carbon:nitrogen ratio (N+P since 1996)

			Aggregate fraction Carbon:Nitrogen			
			Aggregate size fraction			
Treatment	**Date**	**Block**	**>2000 µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1996	Early June	1	23.63	22.44	22.82	19.37
		2	22.04	24.03	24.29	20.65
		3	16.27	24.61	26.30	20.84
		4	19.41	19.08	18.71	15.86
	Mid June	1	21.71	23.38	20.95	19.55
		2	23.31	25.56	26.44	21.31
		3	23.72	22.41	22.62	20.12
		4	18.92	26.46	25.19	19.62
	Late June	1	21.52	24.64	26.50	20.16
		2	24.39	24.52	27.29	21.60
		3	20.04	25.37	18.77	20.61
		4	21.18	27.13	32.94	27.89
	August	1	21.96	22.36	23.36	18.80
		2	25.41	19.21	18.00	18.57
		3	24.15	22.49	20.35	19.85
		4	20.61	11.70	11.19	13.38

Table C9. Aggregate carbon:nitrogen ratio (N+P since 1989)

			Aggregate fraction Carbon:Nitrogen			
			Aggregate size fraction			
Treatment	**Date**	**Block**	**>2000 μm**	**250-2000 μm**	**53-250μm**	**<53μm**
N+P since 1989	Early June	1	24.80	24.29	25.51	22.06
		2	17.54	19.73	20.53	17.18
		3	21.79	28.72	30.96	23.63
		4	20.99	22.49	23.02	19.29
	Mid June	1	23.09	25.15	26.00	22.26
		2	18.25	15.96	17.40	14.85
		3	21.24	15.99	16.77	16.35
		4	22.22	21.73	21.21	18.54
	Late June	1	23.73	21.13	21.30	17.01
		2	22.98*	19.11	18.43	17.07
		3	no sample†	26.58	18.59	20.73
		4	18.91	21.24	21.43	17.56
	August	1	25.25	33.00	32.14	23.01
		2	20.34	15.26	16.16	14.74
		3	19.27	25.14	23.04	18.39
		4	17.55	19.92	20.23	16.25

*No aggregates in sample - only large organic matter. Not used in analysis.

†Not enough sample for carbon analysis.

APPENDIX D

SMALL MACROAGGREGATE SUB-FRACTION CARBON CONTENT,

NITROGEN CONTENT, AND C:N

Table D1. Small macroaggregate sub-fraction carbon content (Control soils)

			Small macroaggregate sub-fraction carbon content (mg C/g fraction)			Small macroaggregate content of small macroaggregate sub-fraction carbon (mg C in sub-fraction/g small macroaggregate)			Whole soil content of small macroaggregate sub-fraction carbon (mg C in sub-fraction/g whole soil)		
			Aggregate size fraction			Aggregate size fraction			Aggregate size fraction		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**
Control	Early June	1	11.04	48.85	63.02	2.07	24.05	20.22	1.25	14.39	12.08
		2	23.80	38.91	40.70	4.27	17.68	14.80	2.22	9.18	7.67
		3	12.97	32.62	43.95	2.55	15.46	15.11	1.42	8.91	8.86
		4	20.00	39.19	41.48	4.08	19.06	13.07	2.12	10.57	7.21
	Mid June	1	26.31	63.52	66.85	6.49	30.45	19.53	3.56	17.40	11.41
		2	36.19	55.98	46.63	5.04	31.55	13.66	2.95	18.19	7.87
		3	11.77	32.97	42.68	2.13	17.39	12.45	1.28	10.48	7.53
		4	10.63	41.22	46.13	1.99	20.57	14.59	1.19	12.24	8.71
	Late June	1	organic soil: Not analyzed			organic soil: Not analyzed			organic soil: Not analyzed		
		2	17.04	36.43	38.98	2.43	21.41	10.50	1.44	12.66	6.21
		3	11.92	37.31	45.17	1.65	21.56	12.82	1.06	13.71	8.12
		4	23.85	33.20	43.60	4.65	18.28	11.09	2.35	9.25	5.61
	August	1	13.09	47.37	60.29	2.56	22.22	20.24	1.39	12.09	11.05
		2	12.51	45.84	41.58	1.98	23.64	13.55	0.86	10.25	5.87
		3	8.45	29.75	45.04	2.08	13.04	14.05	0.72	4.70	5.28
		4	23.89	38.20	40.74	4.07	19.88	12.44	2.17	10.61	6.56

Table D2. Small macroaggregate sub-fraction carbon content (N+P since 1996)

			Small macroaggregate sub-fraction carbon content (mg C/g fraction)			Small macroaggregate content of small macroaggregate sub-fraction carbon (mg C in sub-fraction/g small macroaggregate)			Whole soil content of small macroaggregate sub-fraction carbon (mg C in sub-fraction/g whole soil)		
			Aggregate size fraction			Aggregate size fraction			Aggregate size fraction		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1996	Early June	1	27.51	54.59	52.35	6.07	25.49	16.79	3.29	13.20	8.84
		2	14.97	34.94	49.54	3.56	16.70	14.54	1.97	10.33	8.83
		3	14.04	28.64	36.28	2.55	15.48	10.13	1.42	8.62	5.65
		4	31.34	64.69	51.00	5.71	35.72	13.55	3.79	23.69	8.98
	Mid June	1	22.76	42.23	50.01	4.53	22.85	13.11	2.05	11.07	6.51
		2	14.21	48.60	56.23	3.42	24.48	14.92	1.86	14.19	8.71
		3	12.97	25.47	36.71	3.99	12.08	9.15	1.33	5.37	3.75
		4	21.37	41.95	40.64	5.43	19.78	10.13	2.33	9.13	4.36
	Late June	1	25.37	37.86	43.24	4.54	19.52	13.30	2.46	10.58	7.20
		2	16.45	47.82	61.22	3.45	24.32	17.39	1.96	14.23	10.26
		3	12.96	24.06	37.27	4.28	10.33	8.91	1.43	3.58	3.13
		4	8.33	34.15	46.12	1.92	18.52	10.54	1.04	10.23	5.86
	August	1	22.43	51.72	57.27	3.27	27.79	18.25	1.95	16.67	11.10
		2	8.03	22.85	36.37	2.36	11.25	7.77	1.19	5.64	3.90
		3	7.83	24.46	36.44	1.17	12.24	12.74	0.63	6.53	6.80
		4	9.61	22.45	31.37	3.45	9.44	6.67	1.33	4.78	3.07

Table D3. Small macroaggregate sub-fraction carbon content (N+P since 1989)

			Small macroaggregate sub-fraction carbon content (mg C/g fraction)			Small macroaggregate content of small macroaggregate sub-fraction carbon (mg C in sub-fraction/g small macroaggregate)			Whole soil content of small macroaggregate sub-fraction carbon (mg C in sub-fraction/g whole soil)		
			Aggregate size fraction			Aggregate size fraction			Aggregate size fraction		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1989	Early June	1	16.11	44.81	50.46	3.49	22.98	13.73	1.69	11.22	6.71
		2	15.04	31.51	36.64	2.54	15.91	12.10	1.59	10.24	7.74
		3	21.53	36.48	43.61	4.74	17.51	12.69	2.12	7.80	5.60
		4	30.71	49.36	49.94	4.57	27.18	14.86	2.93	17.57	9.58
	Mid June	1	16.22	46.00	51.79	3.09	24.31	14.91	1.74	14.19	8.97
		2	11.28	30.60	34.28	2.39	15.86	9.41	1.26	8.91	5.02
		3	17.93	36.77	42.69	5.21	16.55	11.18	2.34	7.80	5.17
		4	21.95	45.88	45.33	3.50	25.87	12.63	2.20	16.18	7.85
	Late June	1	28.74	40.85	46.37	6.79	19.49	12.78	3.03	8.51	5.64
		2	40.43	51.60	48.01	10.98	23.32	13.27	4.74	10.07	5.73
		3	15.28	34.18	42.37	3.70	16.63	11.00	1.57	7.13	4.78
		4	22.00	35.34	39.08	4.69	17.50	11.46	2.60	9.64	6.33
	August	1	22.92	50.92	51.14	3.64	27.66	14.41	1.84	13.94	7.27
		2	22.28	26.86	34.24	4.72	12.16	11.23	2.56	6.97	6.18
		3	24.58	48.41	48.11	4.70	25.88	13.60	1.98	12.27	6.68
		4	37.80	62.53	56.37	8.80	29.92	15.81	3.90	13.05	6.86

Table D4. Small macroaggregate sub-fraction nitrogen content (Control soils)

			Small macroaggregate sub-fraction nitrogen content (mg N/g fraction)			Small macroaggregate content of small macroaggregate sub-fraction nitrogen (mg N in sub-fraction/g small macroaggregate)			Whole soil content of small macroaggregate sub-fraction nitrogen (mg N in sub-fraction/g whole soil)		
			Aggregate size fraction			Aggregate size fraction			Aggregate size fraction		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**
Control	Early June	1	0.68	2.18	3.09	0.13	1.07	0.99	0.08	0.64	0.59
		2	1.20	1.98	2.41	0.22	0.90	0.88	0.11	0.47	0.45
		3	0.78	1.50	2.20	0.15	0.72	0.74	0.08	0.41	0.43
		4	0.97	1.85	2.25	0.20	0.90	0.71	0.10	0.50	0.39
	Mid June	1	1.31	3.04	3.46	0.32	1.45	1.01	0.18	0.83	0.59
		2	1.68	2.88	2.82	0.24	1.62	0.83	0.14	0.94	0.48
		3	0.71	1.73	2.34	0.13	0.91	0.68	0.08	0.55	0.41
		4	0.70	1.79	2.31	0.13	0.89	0.74	0.08	0.53	0.44
	Late June	1	organic soil: Not analyzed			organic soil: Not analyzed			organic soil: Not analyzed		
		2	0.90	1.68	2.10	0.13	0.99	0.56	0.08	0.58	0.33
		3	0.74	1.56	2.14	0.10	0.90	0.60	0.07	0.58	0.38
		4	0.96	1.55	2.30	0.19	0.85	0.59	0.09	0.43	0.30
	August	1	0.66	2.00	2.86	0.13	0.94	0.96	0.07	0.51	0.52
		2	0.71	2.38	2.56	0.11	1.23	0.84	0.05	0.53	0.36
		3	0.55	1.43	2.21	0.14	0.63	0.67	0.05	0.23	0.25
		4	1.03	1.84	2.21	0.18	0.96	0.67	0.09	0.51	0.35

Table D5. Small macroaggregate sub-fraction nitrogen content (N+P since 1996)

			Small macroaggregate sub-fraction nitrogen content (mg N/g fraction)			Small macroaggregate content of small macroaggregate sub-fraction nitrogen (mg N in sub-fraction/g small macroaggregate)			Whole soil content of small macroaggregate sub-fraction nitrogen (mg N in sub-fraction/g whole soil)		
			Aggregate size fraction			Aggregate size fraction			Aggregate size fraction		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1996	Early June	1	1.36	2.63	2.93	0.30	1.23	0.94	0.17	0.64	0.50
		2	0.84	1.70	2.65	0.20	0.81	0.78	0.11	0.50	0.47
		3	0.80	1.41	2.04	0.15	0.76	0.57	0.08	0.42	0.32
		4	1.50	3.63	3.41	0.27	2.00	0.91	0.18	1.33	0.60
	Mid June	1	1.15	2.65	2.78	0.23	1.44	0.73	0.10	0.73	0.36
		2	0.86	2.11	2.84	0.20	1.06	0.75	0.11	0.61	0.44
		3	0.82	1.26	2.11	0.24	0.59	0.53	0.08	0.26	0.22
		4	1.10	2.19	2.47	0.30	1.01	0.62	0.12	0.47	0.27
	Late June	1	1.25	1.99	2.55	0.22	1.03	0.78	0.12	0.56	0.42
		2	0.93	2.02	2.85	0.19	1.03	0.81	0.11	0.60	0.47
		3	0.74	1.15	1.92	0.24	0.49	0.46	0.08	0.17	0.16
		4	0.63	1.66	2.46	0.15	0.90	0.56	0.08	0.49	0.31
	August	1	1.07	2.41	3.03	0.16	1.30	0.96	0.09	0.78	0.59
		2	0.51	1.22	2.10	0.15	0.60	0.45	0.07	0.30	0.23
		3	0.44	1.13	1.70	0.07	0.56	0.59	0.04	0.30	0.32
		4	0.61	1.37	2.05	0.23	0.57	0.44	0.08	0.28	0.20

Table D6. Small macroaggregate sub-fraction nitrogen content (N+P since 1989)

			Small macroaggregate sub-fraction nitrogen content (mg N/g fraction)			Small macroaggregate content of small macroaggregate sub-fraction nitrogen (mg N in sub-fraction/g small macroaggregate)			Whole soil content of small macroaggregate sub-fraction nitrogen (mg N in sub-fraction/g whole soil)		
			Aggregate size fraction			Aggregate size fraction			Aggregate size fraction		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1989	Early June	1	0.94	1.97	2.53	0.20	1.01	0.69	0.10	0.49	0.34
		2	1.04	1.78	2.39	0.18	0.90	0.79	0.11	0.57	0.50
		3	1.16	1.70	2.40	0.26	0.82	0.70	0.11	0.37	0.31
		4	1.59	2.38	2.83	0.24	1.31	0.84	0.15	0.85	0.54
	Mid June	1	0.95	2.05	2.53	0.18	1.08	0.73	0.10	0.63	0.44
		2	0.82	1.86	2.32	0.18	0.96	0.64	0.09	0.54	0.34
		3	1.11	1.83	2.31	0.32	0.82	0.61	0.14	0.39	0.28
		4	1.07	2.23	2.64	0.17	1.26	0.73	0.11	0.79	0.46
	Late June	1	1.27	2.11	2.64	0.31	1.01	0.73	0.13	0.44	0.32
		2	1.98	2.94	3.17	0.54	1.33	0.88	0.23	0.57	0.38
		3	0.88	1.62	2.19	0.22	0.79	0.57	0.09	0.34	0.25
		4	1.27	1.90	2.40	0.27	0.94	0.70	0.15	0.52	0.39
	August	1	1.12	2.42	2.65	0.18	1.31	0.75	0.09	0.66	0.38
		2	1.23	1.57	2.16	0.27	0.71	0.71	0.14	0.40	0.39
		3	1.34	2.45	2.73	0.25	1.31	0.77	0.11	0.62	0.38
		4	1.70	3.22	3.40	0.40	1.54	0.95	0.18	0.67	0.41

Table D7 Small macroaggregate sub-fraction carbon:nitrogen ratio (Control soils)

			Small macroaggregate sub-fraction carbon:nitrogen ratio		
			Aggregate size fraction		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**
Control	Early June	1	16.34	22.39	20.42
		2	19.84	19.68	16.87
		3	16.65	21.68	19.94
		4	20.52	21.20	18.46
	Mid June	1	20.10	20.90	19.31
		2	21.49	19.46	16.55
		3	16.58	19.10	18.22
		4	15.13	23.08	19.94
	Late June	1		organic soil: C:N not analyzed	
		2	18.85	21.65	18.60
		3	16.18	23.95	21.12
		4	24.82	21.47	18.94
	August	1	19.72	23.72	21.10
		2	17.57	19.27	16.23
		3	15.40	20.77	20.39
		4	23.13	20.74	18.42

Table D8. Small macroaggregate sub-fraction carbon:nitrogen ratio (N+P since 1996)

			Small macroaggregate sub-fraction carbon:nitrogen ratio		
			Aggregate size fraction		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1996	Early June	1	20.30	20.76	17.85
		2	17.75	20.50	18.67
		3	17.63	20.38	17.74
		4	20.95	17.82	14.95
	Mid June	1	19.77	15.93	17.98
		2	16.58	23.05	19.79
		3	15.77	20.19	17.38
		4	19.49	19.18	16.43
	Late June	1	20.24	19.00	16.97
		2	17.75	23.65	21.46
		3	17.55	20.87	19.40
		4	13.25	20.60	18.74
	August	1	20.89	21.45	18.92
		2	15.89	18.74	17.30
		3	17.87	21.74	21.42
		4	15.82	16.40	15.29

Table D9. Small macroaggregate sub-fraction carbon:nitrogen ratio (N+P since 1989)

			Small macroaggregate sub-fraction carbon:nitrogen ratio		
			Aggregate size fraction		
Treatment	**Date**	**Block**	**250-2000 µm**	**53-250µm**	**<53µm**
N+P since 1989	Early June	1	17.13	22.80	19.91
		2	14.51	17.73	15.32
		3	18.62	21.43	18.21
		4	19.33	20.76	17.64
	Mid June	1	17.09	22.41	20.46
		2	13.83	16.41	14.78
		3	16.22	20.09	18.48
		4	20.57	20.58	17.19
	Late June	1	22.66	19.35	17.55
		2	20.40	17.58	15.16
		3	17.37	21.16	19.33
		4	17.28	18.57	16.27
	August	1	20.52	21.08	19.32
		2	18.10	17.08	15.82
		3	18.40	19.79	17.62
		4	22.25	19.43	16.58

APPENDIX E

WHOLE SOIL CARBON CONTENT, NITROGEN CONTENT, AND C:N

Table E1: Whole soil carbon content, nitrogen, and carbon:nitrogen ratio from Control soils.

Table E2: Whole soil carbon content, nitrogen, and carbon:nitrogen ratio from soils with nutrient addition since 1996.

Table E3: Whole soil carbon content, nitrogen, and carbon:nitrogen ratio from soils with nutrient addition since 1989.

Table E1. Whole soil carbon content, nitrogen content, and carbon:nitrogen ratio (Control soils)

Treatment	Date	Block	Whole Soil Carbon content mg C/g whole soil	Whole Soil Nitrogen content mg N/g whole soil	Whole Soil C:N
Control	Early June	1	52.12	2.15	24.25
		2	39.39	1.79	22.06
		3	36.65	1.47	24.93
		4	36.82	1.48	24.88
	Mid June	1	59.45	2.70	22.03
		2	50.92	2.53	20.12
		3	34.54	1.50	23.07
		4	39.91	1.64	24.35
	Late June	1	organic soil: Not analyzed for C and N		
		2	33.61	1.58	21.24
		3	40.40	1.52	26.49
		4	33.75	1.30	26.01
	August	1	49.61	1.98	25.07
		2	37.44	2.14	17.47
		3	34.66	1.66	20.85
		4	37.85	1.82	20.81

Table E2. Whole soil carbon content, nitrogen content, and carbon:nitrogen ratio (N+P since 1996)

Treatment	Date	Block	Whole Soil Carbon content mg C/g whole soil	Whole Soil Nitrogen content mg N/g whole soil	Whole Soil C:N
N+P since 1996	Early June	1	51.71	2.33	22.20
		2	39.59	1.70	23.32
		3	29.24	1.27	22.99
		4	60.20	3.19	18.85
	Mid June	1	43.77	2.00	21.93
		2	46.44	1.87	24.86
		3	27.12	1.23	21.98
		4	38.19	2.00	19.13
	Late June	1	36.56	1.72	21.31
		2	49.86	1.98	25.23
		3	26.40	1.02	25.88
		4	34.95	1.50	23.28
	August	1	52.14	2.38	21.94
		2	24.42	1.29	18.94
		3	28.44	1.28	22.20
		4	25.16	1.48	16.96

Table E3. Whole soil carbon content, nitrogen content, and carbon:nitrogen ratio (N+P since 1989)

Treatment	Date	Block	Whole Soil Carbon content mg C/g whole soil	Whole Soil Nitrogen content mg N/g whole soil	Whole Soil C:N
N+P since 1989	Early June	1	44.19	1.83	24.17
		2	33.69	1.73	19.43
		3	36.35	1.57	23.08
		4	51.27	2.34	21.94
	Mid June	1	44.96	1.81	24.88
		2	29.23	1.53	19.07
		3	36.21	1.62	22.37
		4	42.37	2.00	21.15
	Late June	1	38.34	1.91	20.03
		2	36.96	1.99	18.58
		3	35.41	1.65	21.43
		4	33.87	1.66	20.34
	August	1	45.03	1.96	23.02
		2	28.84	1.75	16.48
		3	38.69	1.98	19.56
		4	51.05	2.63	19.41

APPENDIX F

CARBON AND NITROGEN CONTENT OF SILT+CLAY (<53 μm) FROM SMALL MACROAGGREGATES AND MICROAGGREGATES

Table F1: Carbon content of silt+clay (<53 μm) from small macroaggregates and microaggregates from control soils.

Table F2: Carbon content of silt+clay (<53 μm) from small macroaggregates and microaggregates from soils with nutrient addition since 1996.

Table F3: Carbon content of silt+clay (<53 μm) from small macroaggregates and microaggregates from soils with nutrient addition since 1989.

Table F4: Nitrogen content of silt+clay (<53 μm) from small macroaggregates and microaggregates from control soils.

Table F5: Nitrogen content of silt+clay (<53 μm) from small macroaggregates and microaggregates from soils with nutrient addition since 1996.

Table F6: Nitrogen content of silt+clay (<53 μm) from small macroaggregates and microaggregates from soils with nutrient addition since 1989.

Table F1. Carbon content of Silt+clay (<53μm) from Small Macroaggregates and Microaggregates (Control Soils)

			Small Macroaggregate (250-2000μm)			Microaggregate (53-250μm)		
Treatment	**Date**	**Block**	**mg total <53μm C from small macro-aggregate/g total <53μm from small macro-aggregate**	**mg small macro-aggregate <53μm subfraction C/g small macro-aggregate**	**mg small macro-aggregate <53μm subfraction C/g whole soil**	**mg total <53μm C from micro-aggregate/g total <53μm from micro-aggregate**	**mg micro-aggregate <53μm subfraction C/g micro-aggregate**	**mg micro-aggregate <53μm subfraction C/g whole soil**
Control	Early June	1	60.29	39.03	23.29	59.08	31.89	5.61
		2	40.13	26.87	13.95	40.70	23.94	6.15
		3	41.57	27.44	16.01	43.42	23.14	4.34
		4	43.45	27.44	15.30	39.63	23.66	5.53
	Mid June	1	68.20	40.80	23.59	58.61	28.75	6.03
		2	49.80	36.19	20.80	44.98	29.62	7.38
		3	41.16	25.86	15.56	39.70	22.13	4.66
		4	46.72	32.19	19.18	43.46	28.10	5.52
	Late June	1	organic soil: Not analyzed for <53 fraction			organic soil: Not analyzed for <53 fraction		
		2	41.58	29.90	17.67	34.15	21.61	5.33
		3	42.36	28.93	18.39	41.69	24.34	4.25
		4	41.93	25.79	13.05	35.99	21.41	6.28
	August	1	55.93	33.15	18.10	57.39	27.96	5.23
		2	44.71	30.44	13.20	34.66	23.17	8.11
		3	43.81	24.14	8.95	39.63	23.48	6.56
		4	40.02	23.95	12.65	36.85	19.42	4.42

Table F2. Carbon content of Silt+clay (<53µm) from Small Macroaggregates and Microaggregates (N+P since 1996)

			Small Macroaggregate (250-2000µm)			Microaggregate (53-250µm)		
Treatment	**Date**	**Block**	**mg total <53µm C from small macro-aggregate/g total <53µm from small macro-aggregate**	**mg small macro-aggregate <53µm subfraction C/g small macro-aggregate**	**mg small macro-aggregate <53µm subfraction C/g whole soil**	**mg total <53µm C from micro-aggregate/g total <53µm from micro-aggregate**	**mg micro-aggregate <53µm subfraction C/g micro-aggregate**	**mg micro-aggregate <53µm subfraction C/g whole soil**
N+P since 1996	Early June	1	52.17	36.22	19.04	52.01	29.58	5.69
		2	49.46	27.33	16.89	44.78	26.22	6.22
		3	33.07	20.36	11.35	33.82	19.01	4.06
		4	58.99	41.61	27.59	56.23	33.33	4.55
	Mid June	1	47.22	28.49	13.92	46.97	30.06	9.09
		2	55.64	33.72	19.64	53.72	32.34	7.86
		3	32.96	16.72	7.41	32.79	17.45	5.78
		4	37.72	19.57	6.73	32.54	18.76	4.48
	Late June	1	40.06	25.62	13.88	40.47	23.14	5.95
		2	61.71	39.08	23.09	57.25	32.27	7.50
		3	34.24	14.93	5.24	35.22	18.25	7.19
		4	42.16	24.21	13.34	40.68	25.61	7.06
	August	1	53.35	36.67	22.16	52.56	31.65	6.53
		2	32.46	16.86	8.47	33.77	18.02	5.61
		3	34.82	21.88	11.67	34.68	19.24	2.86
		4	31.85	13.72	6.62	26.29	13.68	4.01

Table F3. Carbon content of Silt+clay (<53µm) from Small Macroaggregates and Microaggregates (N+P since 1989)

			Small Macroaggregate (250-2000µm)			Microaggregate (53-250µm)		
Treatment	**Date**	**Block**	**mg total <53µm C from small macro-aggregate/g total <53µm from small macro-aggregate**	**mg small macro-aggregate <53µm subfraction C/g small macro-aggregate**	**mg small macro-aggregate <53µm subfraction C/g whole soil**	**mg total <53µm C from micro-aggregate/g total <53µm from micro-aggregate**	**mg micro-aggregate <53µm subfraction C/g micro-aggregate**	**mg micro-aggregate <53µm subfraction C/g whole soil**
N+P since 1989	Early June	1	47.94	30.48	14.88	49.09	30.23	9.64
		2	35.50	24.38	15.67	35.98	23.23	4.96
		3	41.10	26.02	11.56	39.32	24.38	8.33
		4	50.00	37.34	24.16	49.25	30.24	4.87
	Mid June	1	53.51	35.61	20.95	52.18	32.51	8.41
		2	34.49	22.26	12.32	34.60	20.64	5.76
		3	37.02	20.45	9.76	40.62	23.49	7.80
		4	45.10	33.51	20.85	42.01	26.50	5.57
	Late June	1	46.68	24.92	11.08	40.56	23.30	7.58
		2	51.14	29.31	12.65	35.97	18.61	4.85
		3	41.39	23.72	10.18	37.51	22.49	8.03
		4	37.73	24.52	13.50	35.26	18.05	4.01
	August	1	52.16	34.35	17.33	47.16	27.92	8.27
		2	32.87	19.35	10.90	31.73	18.32	4.57
		3	46.01	29.21	14.23	39.22	23.56	5.40
		4	58.08	35.31	15.32	47.52	28.75	8.63

Table F4. Nitrogen content of Silt+clay (<53µm) from Small Macroaggregates and Microaggregates (Control Soils)

Treatment	Date	Block	Small Macroaggregate (250-2000µm)			Microaggregate (53-250µm)		
			mg total <53µm N from small macro-aggregate/g total <53µm from small macro-aggregate	**mg small macro-aggregate <53µm subfraction N/g small macro-aggregate**	**mg small macro-aggregate <53µm subfraction N/g whole soil**	**mg total <53µm N from micro-aggregate/g total <53µm from micro-aggregate**	**mg micro-aggregate <53µm subfraction N/g micro-aggregate**	**mg micro-aggregate <53µm subfraction N/g whole soil**
Control	Early June	1	2.66	1.73	1.03	2.79	1.50	0.27
		2	2.11	1.42	0.74	2.38	1.40	0.37
		3	1.88	1.22	0.71	2.25	1.18	0.23
		4	2.21	1.39	0.77	2.08	1.24	0.29
	Mid June	1	3.65	2.18	1.25	3.24	1.58	0.33
		2	2.93	2.14	1.24	2.61	1.71	0.42
		3	2.08	1.30	0.78	2.12	1.18	0.25
		4	2.05	1.42	0.84	2.21	1.42	0.28
	Late June	1	organic soil: Not analyzed for <53 fraction			organic soil: Not analyzed for <53 fraction		
		2	2.17	1.56	0.92	1.69	1.07	0.26
		3	1.84	1.25	0.80	1.87	1.09	0.19
		4	2.14	1.31	0.67	1.70	1.01	0.29
	August	1	2.59	1.53	0.84	2.74	1.32	0.25
		2	2.70	1.84	0.80	1.93	1.29	0.45
		3	2.07	1.13	0.41	1.84	1.09	0.30
		4	2.17	1.30	0.69	1.95	1.03	0.23

Table F5. Nitrogen content of Silt+clay (<53µm) from Small Macroaggregates and Microaggregates (N+P since 1996)

			Small Macroaggregate (250-2000µm)			Microaggregate (53-250µm)		
Treatment	**Date**	**Block**	**mg total <53µm N from small macro-aggregate/g total <53µm from small macro-aggregate**	**mg small macro-aggregate <53µm subfraction N/g small macro-aggregate**	**mg small macro-aggregate <53µm subfraction N/g whole soil**	**mg total <53µm N from micro-aggregate/g total <53µm from micro-aggregate**	**mg micro-aggregate <53µm subfraction N/g micro-aggregate**	**mg micro-aggregate <53µm subfraction N/g whole soil**
N+P since 1996	Early June	1	2.69	1.87	0.98	2.77	1.58	0.30
		2	2.37	1.31	0.81	2.24	1.31	0.32
		3	1.63	1.00	0.56	1.65	0.92	0.20
		4	3.75	2.65	1.75	3.65	2.16	0.30
	Mid June	1	2.53	1.53	0.75	2.41	1.55	0.46
		2	2.57	1.54	0.88	2.81	1.69	0.41
		3	1.81	0.91	0.40	1.89	0.95	0.38
		4	2.04	1.04	0.35	1.78	1.02	0.26
	Late June	1	2.13	1.36	0.74	2.43	1.39	0.36
		2	2.67	1.69	1.00	2.58	1.46	0.34
		3	1.73	0.74	0.25	1.66	0.86	0.34
		4	2.11	1.22	0.67	2.12	1.34	0.37
	August	1	2.67	1.84	1.11	2.95	1.77	0.37
		2	1.78	0.92	0.46	1.93	1.03	0.32
		3	1.50	0.94	0.50	1.59	0.88	0.13
		4	2.14	0.93	0.45	1.69	0.87	0.26

Table F6. Nitrogen content of Silt+clay (<53µm) from Small Macroaggregates and Microaggregates (N+P since 1989)

			Small Macroaggregate (250-2000µm)			Microaggregate (53-250µm)		
Treatment	**Date**	**Block**	**mg total <53µm N from small macro-aggregate/g total <53µm from small macro-aggregate**	**mg small macro-aggregate <53µm subfraction N/g small macro-aggregate**	**mg small macro-aggregate <53µm subfraction N/g whole soil**	**mg total <53µm N from micro-aggregate/g total <53µm from micro-aggregate**	**mg micro-aggregate <53µm subfraction N/g micro-aggregate**	**mg micro-aggregate <53µm subfraction N/g whole soil**
N+P since 1989	Early June	1	2.26	1.44	0.70	2.34	1.44	0.46
		2	2.13	1.47	0.95	2.16	1.39	0.30
		3	2.08	1.32	0.59	1.96	1.22	0.42
		4	2.53	1.89	1.22	2.74	1.68	0.27
	Mid June	1	2.56	1.70	1.00	2.80	1.75	0.45
		2	2.03	1.31	0.73	2.41	1.44	0.40
		3	2.10	1.24	0.59	2.25	1.29	0.43
		4	2.30	1.71	1.06	2.42	1.53	0.32
	Late June	1	2.51	1.34	0.60	2.08	1.20	0.39
		2	3.36	1.93	0.83	2.10	1.09	0.28
		3	2.08	1.19	0.51	1.72	1.03	0.36
		4	2.25	1.45	0.80	1.94	1.00	0.22
	August	1	2.85	1.89	0.96	2.42	1.43	0.42
		2	2.01	1.18	0.66	2.00	1.16	0.29
		3	2.58	1.64	0.78	2.08	1.24	0.28
		4	3.46	2.10	0.91	2.92	1.77	0.53

APPENDIX G

SMALL MACROAGGREGATE AND MICROAGGREGATE LIGHT FRACTION CARBON CONTENT

Table G1. Small Macroaggregate (250-2000 μm) light fraction carbon content from control soils.

Table G2. Small Macroaggregate (250-2000 μm) light fraction carbon content from soils with nutrient addition since 1996.

Table G3. Small Macroaggregate (250-2000 μm) light fraction carbon content from soils with nutrient addition since 1989.

Table G4. Microaggregate (53-250 μm) light fraction carbon content from control soils.

Table G5. Microaggregate (53-250 μm) light fraction carbon content from soils with nutrient addition since 1996.

Table G6. Microaggregate (53-250 μm) light fraction carbon content from soils with nutrient addition since 1989.

Table G1. Small Macroaggregate (250-2000μm) light fraction carbon content (Control Soils)

			Small Macroaggregate				
Treatment	**Date**	**Block**	**mg LF-C/g LF**	**mg LF-C/g small macroaggregate**	**mg small macroaggregate LF-C/g whole soil**	**% of small macroaggregate C in LF**	**% of total soil C as small macroaggregate LF-C**
Control	Early June	1	263.00	1.80	1.09	3.41	2.04
		2	295.47	3.32	1.74	7.85	4.24
		3	258.86	1.44	0.82	4.10	2.33
		4	249.15	2.49	1.31	6.53	3.50
	Mid June	1	208.05	8.28	4.53	12.23	7.04
		2	263.81	3.70	2.23	6.74	4.24
		3	262.85	1.50	0.87	4.15	2.42
		4	279.00	1.21	0.73	3.13	1.88
	Late June	1	----------------------------organic soil: Not analyzed for light fraction carbon--				
		2	320.07	2.18	1.29	6.19	3.83
		3	271.19	1.23	0.78	3.02	1.97
		4	269.40	2.49	1.26	7.11	3.78
	August	1	262.32	3.23	1.73	5.73	3.08
		2	300.45	1.78	0.77	4.14	2.06
		3	294.78	1.43	0.50	4.63	1.50
		4	283.02	3.47	1.85	7.72	4.15

Table G2. Small Macroaggregate (250-2000μm) light fraction carbon content (N+P since 1996)

			Small Macroaggregate				
Treatment	**Date**	**Block**	**mg LF-C/g LF**	**mg LF-C/g small macroaggregate**	**mg small macroaggregate LF-C/g whole soil**	**% of small macroaggregate C in LF**	**% of total soil C as small macroaggregate LF-C**
N+P since 1996	Early June	1	272.39	4.67	2.43	8.88	4.79
		2	257.61	2.35	1.32	6.30	3.36
		3	280.18	1.76	0.98	6.02	3.36
		4	278.55	3.36	2.23	5.45	3.70
	Mid June	1	201.48	2.41	1.11	5.31	2.53
		2	208.76	2.37	1.26	5.45	2.87
		3	286.61	1.51	0.57	5.82	2.07
		4	299.26	2.75	0.94	8.26	2.45
	Late June	1	289.48	3.79	2.06	9.75	5.63
		2	272.08	2.79	1.55	5.84	3.18
		3	295.72	2.25	0.73	9.79	2.91
		4	279.17	1.58	0.86	4.60	2.48
	August	1	305.70	3.51	2.06	6.25	3.85
		2	335.94	1.47	0.73	6.81	3.00
		3	264.74	1.22	0.65	4.33	2.28
		4	290.72	3.66	1.40	24.58	5.45

Table G3. Small Macroaggregate (250-2000µm) light fraction carbon content (N+P since 1989)

			Small Macroaggregate				
Treatment	**Date**	**Block**	**mg LF-C/g LF**	**mg LF-C/g small macroaggregate**	**mg small macroaggregate LF-C/g whole soil**	**% of small macroaggregate C in LF**	**% of total soil C as small macroaggregate LF-C**
N+P since 1989	Early June	1	333.31	2.06	1.00	4.57	2.25
		2	291.78	1.64	1.05	5.02	3.14
		3	277.54	2.43	1.10	6.78	3.15
		4	293.28	3.47	2.23	6.62	4.35
	Mid June	1	312.07	1.73	1.02	3.80	2.29
		2	323.56	1.71	0.89	6.34	3.09
		3	282.56	2.46	1.16	6.71	2.91
		4	295.27	2.33	1.45	5.35	3.43
	Late June	1	289.78	3.90	1.78	9.47	4.52
		2	256.97	5.61	2.42	10.79	6.55
		3	292.44	2.17	0.94	6.46	2.68
		4	267.68	2.32	1.28	6.65	3.82
	August	1	271.00	3.16	1.60	5.49	3.09
		2	301.93	3.60	2.04	12.46	6.60
		3	280.09	4.07	1.75	7.42	4.43
		4	272.40	8.02	3.55	13.54	6.94

Table G4. Microaggregate (53-250µm) light fraction carbon content (Control Soils)

Treatment	Date	Block	Microaggregate: mg LF-C/g LF	Microaggregate: mg LF-C/g microaggregate	Microaggregate: mg microaggregate LF-C/g whole soil	Microaggregate: % of microaggregate C in LF	Microaggregate: % of total soil C as microaggregate LF-C
Control	Early June	1	246.49	2.99	0.53	6.53	1.01
		2	350.37	0.98	0.25	3.15	0.65
		3	283.67	1.52	0.27	4.78	0.73
		4	300.93	1.06	0.26	3.44	0.69
	Mid June	1	225.49	5.27	1.14	10.12	1.82
		2	309.86	1.36	0.32	3.24	0.64
		3	288.54	0.75	0.13	2.34	0.36
		4	295.16	1.05	0.21	2.95	0.52
	Late June	1	----------organic soil: Not analyzed for light fraction carbon----------				
		2	337.42	1.00	0.25	3.62	0.74
		3	266.43	1.23	0.21	3.42	0.50
		4	310.18	0.81	0.24	2.90	0.69
	August	1	244.43	3.92	0.77	9.61	1.40
		2	392.98	0.77	0.27	2.47	0.72
		3	308.54	1.05	0.29	3.52	0.89
		4	302.29	1.30	0.29	4.34	0.70

Table G5. Microaggregate (53-250μm) light fraction carbon content (N+P since 1996)

			Microaggregate				
Treatment	**Date**	**Block**	**mg LF-C/g LF**	**mg LF-C/g microaggregate**	**mg microaggregate LF-C/g whole soil**	**% of microaggregate C in LF**	**% of total soil C as microaggregate LF-C**
N+P since 1996	Early June	1	318.34	2.22	0.43	4.87	0.79
		2	286.03	1.35	0.31	3.66	0.78
		3	349.38	0.65	0.14	2.78	0.47
		4	291.16	1.48	0.20	3.14	0.34
	Mid June	1	318.04	1.81	0.56	4.72	1.26
		2	288.82	1.25	0.31	3.05	0.71
		3	370.90	1.08	0.37	5.03	1.37
		4	391.22	0.83	0.20	3.43	0.75
	Late June	1	310.19	1.46	0.38	4.89	1.02
		2	278.48	1.54	0.37	3.42	0.77
		3	320.67	1.16	0.46	5.21	1.79
		4	300.87	1.40	0.37	3.92	0.99
	August	1	309.91	1.96	0.44	4.28	0.79
		2	360.07	0.66	0.20	2.85	0.83
		3	288.15	1.00	0.15	4.07	0.52
		4	371.10	0.78	0.24	4.77	1.16

Table G6. Microaggregate (53-250µm) light fraction carbon content (N+P since 1989)

			Microaggregate				
Treatment	**Date**	**Block**	**mg LF-C/g LF**	**mg LF-C/g microaggregate**	**mg microaggregate LF-C/g whole soil**	**% of microaggregate C in LF**	**% of total soil C as microaggregate LF-C**
N+P since 1989	Early June	1	283.13	1.48	0.48	3.61	1.07
		2	336.30	0.68	0.15	2.28	0.45
		3	332.46	1.01	0.35	3.09	0.93
		4	319.13	1.41	0.23	3.53	0.44
	Mid June	1	299.80	1.07	0.27	2.62	0.60
		2	373.15	0.51	0.15	2.21	0.59
		3	318.12	1.51	0.48	4.75	1.30
		4	341.83	0.80	0.17	2.27	0.40
	Late June	1	315.09	1.35	0.40	4.02	1.00
		2	309.95	1.40	0.37	4.50	0.99
		3	328.34	1.18	0.40	3.76	1.13
		4	342.75	1.06	0.24	4.11	0.73
	August	1	341.54	1.37	0.38	3.00	0.75
		2	354.67	0.98	0.24	4.14	0.88
		3	321.85	1.38	0.31	3.80	0.79
		4	296.55	3.09	0.94	6.45	1.83

APPENDIX H

SMALL MACROAGGREGATE AND MICROAGGREGATE

LIGHT FRACTION NITROGEN CONTENT

Table H1. Small Macroaggregate (250-2000 μm) light fraction nitrogen content from control soils.

Table H2. Small Macroaggregate (250-2000 μm) light fraction nitrogen content from soils with nutrient addition since 1996.

Table H3. Small Macroaggregate (250-2000 μm) light fraction nitrogen content from soils with nutrient addition since 1989.

Table H4. Microaggregate (53-250 μm) light fraction nitrogen content from control soils.

Table H5. Microaggregate (53-250 μm) light fraction nitrogen content from soils with nutrient addition since 1996.

Table H6. Microaggregate (53-250 μm) light fraction nitrogen content from soils with nutrient addition since 1989.

Table H1. Small Macroaggregate (250-2000μm) light fraction nitrogen content (Control Soils)

			Small Macroaggregate				
Treatment	**Date**	**Block**	**mg LF-N/g LF**	**mg LF-N/g small macroaggregate**	**mg small macroaggregate LF-N/g whole soil**	**% of small macroaggregate N in LF**	**% of total soil N as small macroaggregate LF-N**
Control	Early June	1	10.168	0.071	0.043	3.191	1.880
		2	12.213	0.133	0.070	7.209	3.791
		3	9.903	0.055	0.032	3.996	2.178
		4	9.469	0.095	0.050	6.463	3.370
	Mid June	1	8.899	0.373	0.202	11.772	6.655
		2	11.311	0.164	0.100	6.053	3.753
		3	10.805	0.060	0.035	3.995	2.266
		4	12.055	0.051	0.031	3.213	1.915
	Late June	1	--------------------------------organic soil: Not analyzed for light fraction nitrogen---------------------------------				
		2	12.810	0.087	0.052	5.275	3.257
		3	10.119	0.045	0.029	2.952	1.912
		4	8.383	0.077	0.039	6.071	3.086
	August	1	9.917	0.123	0.066	5.722	2.866
		2	13.257	0.078	0.034	3.325	1.586
		3	12.038	0.058	0.020	3.820	1.227
		4	12.220	0.154	0.083	7.008	3.806

Table H2. Small Macroaggregate (250-2000μm) light fraction nitrogen content (N+P since 1996)

			Small Macroaggregate				
Treatment	**Date**	**Block**	**mg LF-N/g LF**	**mg LF-N/g small macroaggregate**	**mg small macroaggregate LF-N/g whole soil**	**% of small macroaggregate N in LF**	**% of total soil N as small macroaggregate LF-N**
N+P since 1996	Early June	1	12.917	0.222	0.116	9.544	5.115
		2	10.615	0.097	0.054	6.334	3.199
		3	10.502	0.067	0.037	5.570	2.888
		4	11.763	0.142	0.094	4.390	2.944
	Mid June	1	8.212	0.098	0.046	5.117	2.322
		2	8.385	0.094	0.050	5.484	2.798
		3	12.307	0.064	0.025	5.498	2.014
		4	13.017	0.120	0.041	7.659	2.052
	Late June	1	13.039	0.171	0.093	9.758	5.474
		2	11.515	0.127	0.070	6.824	3.610
		3	11.655	0.092	0.029	11.660	3.144
		4	12.535	0.069	0.037	4.910	2.535
	August	1	12.187	0.136	0.080	5.461	3.316
		2	12.190	0.054	0.027	4.931	2.099
		3	11.019	0.051	0.027	4.050	2.109
		4	13.202	0.166	0.063	19.236	4.171

Table H3. Small Macroaggregate (250-2000μm) light fraction nitrogen content (N+P since 1989)

Treatment	Date	Block	Small Macroaggregate: mg LF-N/g LF	mg LF-N/g small macroaggregate	mg small macroaggregate LF-N/g whole soil	% of small macroaggregate N in LF	% of total soil N as small macroaggregate LF-N
N+P since 1989	Early June	1	14.925	0.090	0.044	4.917	2.418
		2	16.155	0.090	0.057	5.406	3.337
		3	13.702	0.122	0.055	8.307	3.625
		4	13.911	0.164	0.105	7.063	4.529
	Mid June	1	13.394	0.074	0.044	4.100	2.471
		2	15.977	0.085	0.044	6.585	2.996
		3	14.395	0.183	0.080	12.198	2.893
		4	12.594	0.100	0.062	4.970	3.097
	Late June	1	11.229	0.143	0.064	7.428	3.343
		2	13.264	0.290	0.125	10.644	6.283
		3	12.668	0.094	0.040	7.430	2.518
		4	13.612	0.118	0.065	7.105	3.902
	August	1	11.908	0.148	0.074	5.754	3.205
		2	15.462	0.183	0.103	10.820	5.525
		3	14.398	0.189	0.083	7.015	4.110
		4	12.310	0.369	0.164	12.506	6.307

Table H4. Microaggregate (53-250μm) light fraction nitrogen content (Control Soils)

			Microaggregate				
Treatment	**Date**	**Block**	**mg LF-N/g LF**	**mg LF-N/g microaggregate**	**mg microaggregate LF-N/g whole soil**	**% of microaggregate N in LF**	**% of total soil N as microaggregate LF-N**
Control	Early June	1	8.232	0.101	0.018	5.397	0.818
		2	11.112	0.031	0.008	2.198	0.447
		3	9.026	0.048	0.008	3.757	0.573
		4	9.026	0.031	0.007	2.694	0.497
	Mid June	1	9.610	0.229	0.050	9.879	1.737
		2	10.854	0.049	0.011	2.491	0.450
		3	9.492	0.025	0.004	1.819	0.282
		4	7.806	0.027	0.005	1.927	0.331
	Late June	1	--------------------------------organic soil: Not analyzed for light fraction nitrogen---------------------------------				
		2	9.861	0.029	0.007	2.387	0.457
		3	7.686	0.034	0.006	2.813	0.372
		4	5.959	0.017	0.005	1.702	0.375
	August	1	7.625	0.140	0.028	8.183	1.187
		2	11.196	0.022	0.008	1.274	0.356
		3	8.963	0.033	0.007	2.290	0.432
		4	10.235	0.049	0.011	3.299	0.513

Table H5. Microaggregate (53-250µm) light fraction nitrogen content (N+P since 1996)

			Microaggregate				
Treatment	**Date**	**Block**	**mg LF-N/g LF**	**mg LF-N/g microaggregate**	**mg microaggregate LF-N/g whole soil**	**% of microaggregate N in LF**	**% of total soil N as microaggregate LF-N**
N+P since 1996	Early June	1	11.402	0.085	0.016	4.093	0.651
		2	9.730	0.046	0.011	3.007	0.625
		3	13.573	0.023	0.005	2.663	0.392
		4	12.340	0.063	0.009	2.493	0.268
	Mid June	1	9.012	0.052	0.016	2.966	0.792
		2	7.835	0.034	0.009	2.181	0.480
		3	8.018	0.026	0.010	3.072	0.900
		4	12.578	0.027	0.006	2.198	0.420
	Late June	1	10.933	0.052	0.013	3.952	0.771
		2	7.150	0.039	0.010	2.403	0.495
		3	8.251	0.030	0.012	3.710	1.229
		4	9.253	0.040	0.011	2.848	0.678
	August	1	9.759	0.064	0.014	3.200	0.562
		2	10.721	0.020	0.006	1.583	0.470
		3	8.481	0.029	0.004	2.440	0.341
		4	13.871	0.029	0.009	3.073	0.688

Table H6. Microaggregate (53-250µm) light fraction nitrogen content (N+P since 1989)

			Microaggregate				
Treatment	**Date**	**Block**	**mg LF-N/g LF**	**mg LF-N/g microaggregate**	**mg microaggregate LF-N/g whole soil**	**% of microaggregate N in LF**	**% of total soil N as microaggregate LF-N**
N+P since 1989	Early June	1	7.474	0.038	0.012	2.383	0.660
		2	13.247	0.025	0.005	1.823	0.342
		3	10.354	0.030	0.010	2.266	0.633
		4	12.116	0.054	0.009	3.114	0.373
	Mid June	1	7.753	0.028	0.007	1.765	0.388
		2	13.710	0.020	0.006	2.118	0.650
		3	10.347	0.049	0.015	3.480	0.831
		4	10.880	0.026	0.005	1.532	0.267
	Late June	1	10.249	0.048	0.014	3.143	0.737
		2	12.086	0.055	0.014	3.233	0.716
		3	9.486	0.034	0.012	2.339	0.702
		4	12.666	0.039	0.009	3.208	0.529
	August	1	10.861	0.047	0.013	2.376	0.548
		2	14.356	0.040	0.010	2.723	0.578
		3	12.210	0.054	0.012	2.966	0.599
		4	12.277	0.130	0.040	5.481	1.502

APPENDIX I

CONCENTRATION OF SMALL MACROAGGREGATE AND

MICROAGGREGATE LIGHT FRACTION MASS AND C:N

Table I1. Concentration of small macroaggregate and microaggregate light fraction mass and carbon:nitrogen from control soils.

Table I2. Concentration of small macroaggregate and microaggregate light fraction mass and carbon:nitrogen from soils with nutrient addition since 1996.

Table I3. Concentration of small macroaggregate and microaggregate light fraction mass and carbon:nitrogen from soils with nutrient addition since 1989

Table I1. Concentration of small macroaggregate and microaggregate light fraction mass and light fraction carbon:nitrogen ratio (Control Soils)

			Small Macroaggregate			Microaggregate		
Treatment	**Date**	**Block**	**% of small macro-aggregate mass as LF**	**% of whole soil mass as small macro-aggregate LF**	**Small Macro-aggregate C:N**	**% of micro-aggregate mass as LF**	**% of whole soil mass as micro-aggregate LF**	**Micro-aggregate C:N**
Control	Early June	1	0.66	0.40	25.86	1.21	0.21	29.94
		2	1.13	0.59	24.19	0.28	0.07	31.53
		3	0.56	0.32	26.14	0.57	0.10	31.43
		4	1.26	0.67	26.31	0.36	0.09	33.34
	Mid June	1	3.90	2.16	23.38	2.41	0.52	23.46
		2	1.46	0.89	23.32	0.44	0.11	28.55
		3	0.55	0.32	24.33	0.29	0.05	30.40
		4	0.43	0.26	23.14	0.37	0.07	37.81
	Late June	1	----organic soil: Not analyzed for light fraction----					
		2	0.68	0.40	24.99	0.30	0.07	34.22
		3	0.46	0.29	26.80	0.47	0.08	34.66
		4	0.92	0.47	32.14	0.27	0.08	52.05
	August	1	1.26	0.68	26.45	1.69	0.33	32.06
		2	0.59	0.26	22.66	0.19	0.07	35.10
		3	0.48	0.17	24.49	0.35	0.09	34.42
		4	1.22	0.65	23.16	0.42	0.09	29.54

Table I2. Concentration of small macroaggregate and microaggregate light fraction mass and light fraction carbon:nitrogen ratio (N+P since 1996)

			Small Macroaggregate			Microaggregate		
Treatment	**Date**	**Block**	**% of small macro-aggregate mass as LF**	**% of whole soil mass as small macro-aggregate LF**	**Small Macro-aggregate C:N**	**% of micro-aggregate mass as LF**	**% of whole soil mass as micro-aggregate LF**	**Micro-aggregate C:N**
N+P since 1996	Early June	1	1.74	0.90	21.09	0.70	0.14	27.92
		2	0.88	0.50	24.27	0.47	0.10	29.40
		3	0.63	0.35	26.68	0.19	0.04	25.74
		4	1.21	0.80	23.68	0.51	0.07	23.59
	Mid June	1	1.20	0.57	24.53	0.57	0.18	35.29
		2	1.05	0.57	24.90	0.43	0.11	36.86
		3	0.52	0.20	23.29	0.29	0.10	46.26
		4	0.97	0.34	22.99	0.22	0.05	31.10
	Late June	1	1.31	0.71	22.20	0.47	0.12	28.37
		2	1.05	0.58	23.63	0.54	0.13	38.95
		3	0.76	0.25	25.37	0.36	0.14	38.86
		4	0.58	0.32	22.27	0.54	0.14	32.52
	August	1	1.14	0.67	25.08	0.64	0.14	31.76
		2	0.44	0.22	27.56	0.18	0.06	33.58
		3	0.46	0.25	24.03	0.35	0.05	33.98
		4	1.26	0.48	22.02	0.21	0.07	26.75

Table I3. Concentration of small macroaggregate and microaggregate light fraction mass and light fraction carbon:nitrogen ratio (N+P since 1989)

			Small Macroaggregate			Microaggregate		
Treatment	**Date**	**Block**	**% of small macro-aggregate mass as LF**	**% of whole soil mass as small macro-aggregate LF**	**Small Macro-aggregate C:N**	**% of micro-aggregate mass as LF**	**% of whole soil mass as micro-aggregate LF**	**Micro-aggregate C:N**
N+P since 1989	Early June	1	0.62	0.30	22.33	0.52	0.17	37.88
		2	0.58	0.37	18.06	0.21	0.04	25.39
		3	0.88	0.39	20.26	0.31	0.11	32.11
		4	1.21	0.78	21.08	0.44	0.07	26.34
	Mid June	1	0.55	0.33	23.30	0.36	0.09	38.67
		2	0.53	0.28	20.25	0.14	0.04	27.22
		3	0.92	0.44	19.63	0.49	0.16	30.74
		4	0.79	0.49	23.45	0.24	0.05	31.42
	Late June	1	1.37	0.63	25.81	0.44	0.13	30.74
		2	2.18	0.94	19.37	0.45	0.12	25.65
		3	0.74	0.32	23.09	0.36	0.12	34.61
		4	0.87	0.48	19.66	0.31	0.07	27.06
	August	1	1.21	0.61	22.76	0.43	0.12	31.45
		2	1.20	0.67	19.53	0.27	0.07	24.71
		3	1.46	0.63	19.45	0.43	0.10	26.36
		4	2.90	1.28	22.13	1.04	0.32	24.15

APPENDIX J

SMALL MACROAGGREGATE AND MICROAGGREGATE INTRA-AGGREGATE PARTICULATE ORGANIC MATTER (iPOM) CARBON CONTENT

Table J1. Small Macroaggregate (250-2000 μm) iPOM carbon content from control soils.

Table J2. Small Macroaggregate (250-2000 μm) iPOM carbon content from soils with nutrient addition since 1996.

Table J3. Small Macroaggregate (250-2000 μm) iPOM carbon content from soils with nutrient addition since 1989.

Table J4. Microaggregate (53-250 μm) iPOM carbon content from control soils.

Table J5. Microaggregate (53-250 μm) iPOM carbon content from soils with nutrient addition since 1996.

Table J6. Microaggregate (53-250 μm) iPOM carbon content from soils with nutrient addition since 1989.

Table J1. Small Macroaggregate (250-2000μm) iPOM carbon content (Control Soils)

			Small Macroaggregate			
Treatment	**Date**	**Block**	**mg iPOM C/g small macroaggregate**	**mg small macroaggregate iPOM C/g whole soil**	**% of small macroaggregate C in iPOM**	**% of total soil C as small macroaggregate iPOM C**
Control	Early June	1	10.41	6.20	20.40	11.91
		2	10.63	5.52	25.92	13.86
		3	7.61	4.30	22.09	12.42
		4	7.37	3.99	19.43	10.73
	Mid June	1	11.91	6.87	19.76	11.75
		2	14.20	8.23	26.74	16.19
		3	6.96	4.25	20.93	12.68
		4	6.59	3.94	16.68	10.01
	Late June	1	---------------------organic soil: Not analyzed for iPOM---------------------------			
		2	3.14	1.86	8.92	5.52
		3	10.96	6.90	26.21	16.94
		4	6.98	3.53	19.72	10.44
	August	1	12.76	7.01	25.52	13.96
		2	10.72	4.65	24.97	12.41
		3	6.62	2.37	21.62	7.23
		4	11.62	6.29	25.46	14.09

Table J2. Small Macroaggregate (250-2000μm) iPOM carbon content (N+P since 1996)

			Small Macroaggregate			
Treatment	**Date**	**Block**	**mg iPOM C/g small macroaggregate**	**mg small macroaggregate iPOM C/g whole soil**	**% of small macroaggregate C in iPOM**	**% of total soil C as small macroaggregate iPOM C**
Control	Early June	1	11.41	6.02	21.91	12.00
		2	9.31	5.44	24.84	13.86
		3	7.04	3.92	24.51	13.60
		4	16.64	11.03	27.01	18.33
	Mid June	1	13.80	6.62	30.57	15.12
		2	10.42	6.01	22.44	12.73
		3	7.96	2.58	31.29	9.70
		4	11.44	3.89	34.79	10.25
	Late June	1	9.69	5.25	24.89	14.38
		2	7.44	4.42	15.02	8.78
		3	7.76	2.70	31.11	9.93
		4	8.77	4.76	25.68	13.85
	August	1	13.68	8.23	25.45	15.96
		2	3.35	1.69	15.41	6.88
		3	5.03	2.68	17.89	9.44
		4	5.97	3.24	16.67	9.77

Table J3. Small Macroaggregate (250-2000μm) iPOM carbon content (N+P since 1989)

			Small Macroaggregate			
Treatment	**Date**	**Block**	**mg iPOM C/g small macroaggregate**	**mg small macroaggregate iPOM C/g whole soil**	**% of small macroaggregate C in iPOM**	**% of total soil C as small macroaggregate iPOM C**
Control	Early June	1	12.17	5.91	27.06	13.33
		2	7.69	4.90	23.56	14.64
		3	8.11	3.53	21.82	9.76
		4	11.54	7.43	22.01	14.48
	Mid June	1	8.17	4.83	17.98	10.82
		2	5.57	2.99	19.20	9.82
		3	10.88	4.56	35.16	13.24
		4	7.97	5.04	17.66	11.57
	Late June	1	11.61	4.96	29.16	13.01
		2	17.07	7.37	32.84	19.94
		3	7.69	3.31	22.90	9.45
		4	8.41	4.66	24.45	14.07
	August	1	11.86	5.96	23.78	12.88
		2	5.61	3.06	20.31	10.34
		3	13.76	6.45	28.51	16.60
		4	14.96	6.48	25.77	12.56

Table J4. Microaggregate (53-250μm) iPOM carbon content (Control Soils)

			Microaggregate			
Treatment	**Date**	**Block**	**mg iPOM C/g microaggregate**	**mg microaggregate iPOM C/g whole soil**	**% of microaggregate C in iPOM**	**% of total soil C as microaggregate iPOM C**
Control	Early June	1	10.86	1.92	23.64	3.66
		2	6.37	1.51	19.33	3.71
		3	5.56	0.95	16.82	2.52
		4	5.75	1.29	19.16	3.55
	Mid June	1	16.73	3.54	32.90	5.93
		2	12.74	3.03	27.64	5.66
		3	6.95	1.48	24.67	4.49
		4	6.34	1.29	18.06	3.30
	Late June	1	--------------------organic soil: Not analyzed for iPOM--------------------------			
		2	5.01	1.24	18.14	3.68
		3	8.83	1.54	25.77	3.88
		4	5.15	1.50	19.15	4.47
	August	1	8.31	1.45	20.80	3.19
		2	7.02	2.46	22.67	6.56
		3	5.14	1.52	17.53	4.69
		4	6.26	1.37	22.46	3.79

Table J5. Microaggregate (53-250μm) iPOM carbon content (N+P since 1996)

			Microaggregate			
Treatment	**Date**	**Block**	**mg iPOM C/g microaggregate**	**mg microaggregate iPOM C/g whole soil**	**% of microaggregate C in iPOM**	**% of total soil C as microaggregate iPOM C**
Control	Early June	1	11.54	2.25	24.72	4.04
		2	7.88	1.66	21.04	4.14
		3	3.66	0.78	14.78	2.53
		4	12.21	1.67	25.96	2.77
	Mid June	1	6.27	1.84	16.80	4.28
		2	7.95	1.98	19.26	4.42
		3	3.15	1.02	14.44	3.80
		4	4.25	1.15	19.57	4.95
	Late June	1	5.16	1.33	17.00	3.58
		2	11.33	2.46	25.00	4.94
		3	3.25	1.27	13.95	4.72
		4	5.41	1.51	17.04	4.43
	August	1	10.61	2.29	23.43	4.19
		2	4.50	1.40	19.41	5.74
		3	4.23	0.63	17.29	2.21
		4	4.33	1.22	21.53	4.79

Table J6. Microaggregate (53-250µm) iPOM carbon content (N+P since 1989)

			Microaggregate			
Treatment	**Date**	**Block**	**mg iPOM C/g microaggregate**	**mg microaggregate iPOM C/g whole soil**	**% of microaggregate C in iPOM**	**% of total soil C as microaggregate iPOM C**
Control	Early June	1	8.33	2.69	20.59	5.99
		2	5.79	1.21	18.64	3.53
		3	6.60	2.26	20.61	6.08
		4	8.51	1.38	21.14	2.68
	Mid June	1	7.49	2.01	18.04	4.39
		2	3.58	0.93	13.52	3.05
		3	6.75	1.94	18.35	5.11
		4	8.04	1.71	22.75	4.09
	Late June	1	7.41	2.48	23.40	6.60
		2	11.18	2.91	35.84	7.88
		3	8.27	2.86	26.15	8.01
		4	6.95	1.55	26.68	4.63
	August	1	10.52	2.96	24.81	6.36
		2	4.23	1.01	17.19	3.67
		3	10.11	2.26	27.03	5.71
		4	16.33	4.92	33.94	9.61

APPENDIX K

LIVE ROOT BIOMASS FROM 8-mm-SIEVED SOIL

Table K1. Live root biomass from 8-mm-sieved soil.

Table K1. Live root biomass from 8mm-sieved soil

			Control Soils	N+P since 1996	N+P since 1989
Treatment	Date	Block	mg dried roots/g dry soil	mg dried roots /g dry soil	mg dried roots/g dry soil
Control	Early June	1	0.075	1.349	1.103
		2	0.380	0.431	0.151
		3	0.735	0.359	3.389
		4	0.685	2.470	3.797
	Mid June	1	1.316	2.040	0.492
		2	0.550	0.274	0.586
		3	1.295	1.076	1.420
		4	0.124	4.321	3.159
	Late June	1	organic soil: Not analyzed for root biomass	5.612	1.553
		2	0.630	0.365	1.487
		3	0.100	0.944	2.555
		4	1.236	3.111	4.961
	August	1	0.212	1.484	0.614
		2	0.360	0.207	0.122
		3	0.359	0.044	1.457
		4	0.077	0.370	0.308