Chapter 1

General Introduction
1.1 Climate change and northern peatlands

Ongoing increases in the atmospheric concentrations of CO$_2$ and other greenhouse gases are leading to rapid changes in the global climate (IPCC, 2007). Climate plays a fundamental role in controlling important hydrological and ecological processes, and there is concern that these processes will be disrupted by climate change, with consequences for the provision of ecosystem services (Costanza et al., 1997; Schroter et al., 2005). It is therefore important to increase our understanding of how Earth’s biological and atmospheric systems behave and interact under different climate scenarios in order to make realistic predictions of the long-term effects of climate change, and thereby develop appropriate and effective adaption and mitigation strategies.

Understanding the response of ecological systems under climate change is also an important test of ecology as a predictive science. As a discipline, ecology is periodically criticized for its tendency to accumulate anecdotal cases and conceptual schemes, at the expense of progress towards synthesis, generalization and predictive power (Peters, 1980; Weiner, 1995; Sagoff, 2003). Ecosystem responses and feedbacks to climate change will be mediated by changes in patterns of the abundance and distribution of organisms, and by alterations to the stocks and flows of matter and energy within and across ecosystems. These phenomena are ecologists’ primary objects of study. For this reason, along with the huge importance of climate to human society, climate change research offers a grand challenge and unifying theme which has the potential to catalyze the development of a “truly predictive science of the biosphere” (Sugden, 1992; Moorcroft, 2006).

There is extensive variation in the predicted magnitude and direction of future climatic changes across different geographic regions (Christensen et al., 2007), with the strongest impacts expected at higher latitudes (ACIA, 2004). Moreover, at higher latitudes, and in northern peatlands in particular, cycles of nutrients, energy and water are expected to be disrupted by climate change (Gorham, 1991; Freeman et al., 1993; Callaghan & Jonasson, 1995; Chapin et al., 2000). In northern peatlands, the generally low rate of primary production of biomass by plants has exceeded the even lower rate of decomposition over the past centuries (Wieder et al., 2006), leading to a slow but important long-term accumulation of organic matter in the soil. Although rainforests are often referred to as the “lungs of the Earth” — peatlands are arguably just as important in the Earth system due to their role as a “carbon sink” which buffers atmospheric CO$_2$ concentrations, thereby influencing the greenhouse activity of the Earth’s atmosphere (Frolking & Roulet, 2007). Since the last deglaciation approximately 12,000 years ago, northern peatlands (concentrated in northern North America,
Fennoscandia and the northern edge of the Eurasian continent) have sequestered an estimated 270 – 370 Pg of carbon (C) in the form of incompletely decomposed plant matter (Adams et al., 1990; Turunen et al., 2002). This is equivalent to 34 – 46% of the size of the atmospheric CO$_2$ pool (Limpens et al., 2008).

High latitude peatlands accumulate carbon due to an imbalance between the rate of uptake by plant primary production, and the rate of decomposition by soil heterotrophic microorganisms. Relative to other biomes, both of these processes are very slow (Vasander & Kettunen, 2006), such that relatively small changes in the rates of either of could have potentially large effects on the overall C balance of the system. There is particular concern that decomposition processes may be more sensitive to climate change than plant productivity (Gorham, 1991; Vitt & Wieder, 2006). Decomposition in northern peatlands is limited by a combination of low temperatures, low pH, low soil oxygen levels and high concentrations of phenolic compounds (Freeman et al., 2001; Moore & Basiliko, 2006). This combination of conditions limits the activity of microorganisms and the enzymes they produce to break down organic matter. Importantly, all of these constraints on decomposition are in some way controlled by climate, either through temperature or hydrology. Climate change could therefore potentially relax these constraints on decomposition, and consequently lead to the status of northern peatlands switching from C sink to C source. Such a scenario could create positive feedbacks to climate warming, with potentially drastic effects on global climate (Woodwell et al., 1998; Schlesinger & Andrews, 2000). Indeed, there is evidence that increased atmospheric CO$_2$, warming and changes in peatland hydrology due to altered precipitation and evapotranspiration leads to increased rates of decomposition, as detected in both higher greenhouse gas emissions and/or increased export of dissolved organic carbon (DOC) (Freeman et al., 2004; Fenner et al., 2007a; Fenner et al., 2007b; Dorrepaal et al., 2009). Given the important role of northern peatlands in global biogeochemistry and their particular sensitivity to climate change, increasing our understanding of the controls over the C balance, and particularly decomposition, in these systems is a critical research priority.

1.2 The importance of understanding peatland nitrogen cycles

Although much of the focus of research on ecosystem responses to climate change has been on carbon cycle processes (due to the important role of CO$_2$ and CH$_4$ as greenhouse gases), these processes are in turn strongly influenced by interactions
with other elemental cycles (Schlesinger, 1997; Finzi et al., 2011). Due to the fundamental constraints on the elemental composition of the major biological macromolecules (carbohydrates, proteins and nucleic acids), biological processes are subject to varying degrees of stoichiometric control. In other words, the biogeochemical transformations involving organic C are intimately linked to the relative availability of other essential biological elements, in particular nitrogen (N) and phosphorous (P) (Sterner & Elser, 2002). For example, the availability of N and P to plants is the main limitation on primary production in many ecosystems (Vitousek & Howarth, 1991); litter quality (C:N and C:P ratios) is a primary driver of differences in rates of litter decomposition (Aerts, 1997; Cornwell et al., 2008; Weedon et al., 2009); and progressive N limitation may constrain the plant productivity response to rising atmospheric CO₂ levels (Hungate et al., 2003; Luo et al., 2004; Luo et al., 2006). In northern peatlands, nitrogen may have a particularly important role in controlling C cycle processes. Both production and decomposition in northern peatlands are generally extremely N-limited (Roswall & Granhall, 1980; Aerts et al., 1992; Aerts et al., 2001; Aerts et al., 2006a; Limpens et al., 2006). Evidence from N deposition gradients suggests that increased N inputs to peatlands alters the growth and species composition of plant communities (Breeuwer et al., 2008), but can also accelerate losses of C due to CO₂ emissions and DOC exports (Bragazza et al., 2006). However, little is known about the response of internal peatland N cycling to climate change — and this is an important knowledge gap in our ability to understand and predict the future of the peatland C sink.

Northern peatlands typically contain relatively large amounts of organic N, but the limitations on decomposition that lead to C storage also mean that the supply of mineral N available to plants and decomposing microorganisms is very low (Roswall & Granhall, 1980; Limpens et al., 2006). Small external inputs through atmospheric deposition and microbial N fixation are important for balancing losses of N from the active layer via stabilization in deeper, long residence-time layers of the peat profile (Roswall & Granhall, 1980), but for the most part both primary producers and decomposers rely on internal cycling processes for the bulk of their N requirements (Bragazza & Limpens, 2004). Due to the prevailing acidic conditions in peatlands, ammonia oxidation and therefore the nitrification/denitrification pathway is generally a very small fraction of the total N budget. Indeed, in the typology of soil nitrogen economies suggested by Schimel and Bennett (2004), peatlands are generally considered to belong to the low-N case where uptake (by both plants and microbes) of low molecular weight organic N compounds predominates (Lipson & Näsholm, 2001; Krab et al., 2008), full mineralization and nitrification is minimal (Roswall & Granhall, 1980), and immobilization by the microbial biomass is a strong sink
for available N (Aerts et al., 2006a). These characteristics suggest that the peatland nitrogen economy (Figure 1-1) is predominantly controlled by the depolymerization of complex organic forms of N, and the dynamics of mobilization and immobilization by the microbial biomass (Schimel & Bennett, 2004), unlike other systems where mineralization and the nitrification/denitrification pathway play more important roles. Understanding N availability under climate change therefore requires knowledge about the dynamics of depolymerization and microbial immobilization under changing environmental conditions.

1.3 Towards a mechanistic understanding of peatland N cycles

Predicting the response of peatland N cycling to climate change requires knowledge of the underlying mechanisms. Measuring stocks and flows of different forms of N
under changing conditions provides information about what kinds of changes can be expected, and therefore phenomenological understanding, but prediction and generalization to novel contexts requires additional mechanistic understanding of the relevant factors driving the observed processes. In the context of N cycling in peatlands, this implies examining the drivers of N transformations in the soil, and the biotic and abiotic interactions that determine and regulate these drivers. As mentioned above, organic N cycling in peatlands is primarily driven by the interplay between depolymerization of complex organic N and the uptake and release of the resulting dissolved organic N by soil microorganisms and plants. The depolymerization is catalyzed by extracellular enzymes such as proteases, peptidase and ureases (Tate, 2002) and in turn these enzymes are produced by soil microorganisms. If we assume that there is some degree of functional specialization between different groups of microorganisms, then the pools of enzymes can potentially be controlled by the community composition and/or structure of soil microorganisms. Combining these basic ideas leads to a simple conceptual model of the relationships between transformations, enzyme pools, and soil microbes (Figure 1-2). Our conceptual model predicts that climate change related shifts in the rates of organic N transformations in peatlands will be to some extent mediated by changes in the activity of soil extracellular enzymes, and/or the composition of the soil microbial community that produces them. This implies a cascade of control from microbes, to enzymes to N transformations. There is also the potential for indirect effects on each of these three compartments, acting via climate-related changes to the composition and/or productivity of the overlying plant community. Moreover, there is the potential for feedbacks between the different levels of organization, for example due to economic regulation of enzyme production by microbes, or shifts in the composition of the microbial community due to different nutrient availabilities. In the rest of this section I briefly review the reasoning behind focussing on soil enzymes and microbial community composition in order to understand climate change effects on peatland N cycling.

1.3.1 Extracellular enzyme activities

The proximate drivers of the transformations of nitrogen from complex organic forms to assimilable low molecular weight or inorganic forms are extracellular enzymes produced by soil microorganisms (Nannipieri et al., 2002; Allison et al., 2007). These enzymes catalyze the hydrolytic or oxidative reactions that breakdown polymeric compounds into their component monomers. A wide range of enzymes, each specialized for the breakdown of specific classes of substrates are produced by microorganisms (Skujinš, 1976), and the relative levels of production and activity of
Figure 1-2: Conceptual scheme of the causal relationships between components of the soil organic nitrogen cycle. Boxes represent processes or states and linking arrows represent potential causal relationships between them. Grey boxes represent variables which are directly measured in the research described in this thesis. Climate change is considered a driving variable and is experimentally simulated in most of the studies. Plant community composition and productivity is potentially important but not directly measured or manipulated in the studies of this thesis.

these different classes of enzymes are indicators of the physiological function and nutrient status of the soil microbial community (Allison & Vitousek, 2005; Allison et al., 2011). Indeed, given their relatively rapid turnover in soils, they may provide more fine-scale temporal resolution of soil nutrient dynamics than measurements of soil nutrient pools, which reflect the net outcome of multiple processes integrated over time. Methods for measuring the activity of soil enzymes are well developed (Freeman et al., 1995; Wallenstein & Weintraub, 2008), and can be applied to a large number of samples simultaneously. They have been used extensively to investigate the responses of soil nutrient cycling to changes in environmental conditions and substrate availabilities (Allison & Vitousek, 2005; Wallenstein et al., 2009; Henry, 2012) and have revealed important insights into the functioning of peatland ecosys-
tems (Freeman et al., 2001; Fenner et al., 2005; Bonnett et al., 2006). In recent years, there has been particular interest in the utility of soil enzyme-based approaches to help understand and model soil C and N dynamics (Sinsabaugh & Moorhead, 1994; Schimel & Weintraub, 2003; Allison et al., 2010a), and improve the biological realism of models of soil processes in the large-scale Earth Systems Models used to make climate change projections (Todd-Brown et al., 2012). Soil enzymes measurements therefore seem to offer great potential for providing deeper insights into the links between microbial dynamics and peatland N cycles.

1.3.2 Molecular Microbial Ecology

Microorganisms play a central role in driving soil functions and their response to changing climate will be important in determining the direction and magnitude of terrestrial ecosystem feedbacks to climate (Bardgett et al., 2008; Singh et al., 2010). This is especially true for N cycles, as the majority of enzymes that control the different components of the soil N cycle are produced by microorganisms. Recent advances in molecular microbial ecology have furthered our appreciation of the enormous taxonomic and functional diversity of soil-borne microbial communities (Roesch et al., 2007). The recognition of this phylogenetic and functional diversity, and the importance of this diversity for global biogeochemical cycles (Falkowski et al., 2008) has lead to a large research effort dedicated to understanding the controls of microbial community composition, and how this affects biogeochemical cycling and other important ecosystem functions (Fuhrman, 2009). It has been proposed that the response of soil ecosystem processes, such as decomposition and trace gas emissions, to climate change will be mediated by changes in the structure and composition of the soil microbial community (Schimel & Gulledge, 1998; Allison & Martiny, 2008). This proposal is underpinned by the hypothesis of a direct link between the phylogenetic and/or functional potential (presence and/or transcription of functional genes) in a given environment, and the rate or magnitude of the ecosystem process of interest (Fierer et al., 2007; He et al., 2007; Yergeau et al., 2010). However, evidence for such a link is contested, and even if it exists, our ability to detect it is compromised by daunting methodological and conceptual difficulties (Forney et al., 2004; Kowalchuk et al., 2007; Prosser, 2012). Nevertheless, rapid advances in the development of high-throughput, high-resolution methods for profiling microbial communities are removing at least some of these constraints (Caporaso et al., 2011). Northern peatland microbial communities are dominated by bacteria, chiefly members of the phyla Acidobacteria, Actinobacteria and the classes Alphaproteobacteria and Gammaproteobacteria (Pankratov et al., 2005; Dedysh et al., 2006; Pankratov et al., 2006;
Pankratov et al., 2008; Tveit et al., 2013). However, hardly anything is known about how community composition of peatlands is affected by climate changes, and if there is any relation between the relative abundance of different bacterial taxa and the dynamics of C and N cycling. An integrative approach that links profiling of peatland microbial communities with measurements of enzyme activities and N fluxes has the potential to yield important insights into not only the functioning of peatland ecosystems, but also the general relationship between microbial community composition and ecosystem function.

1.4 Aims and Research Questions

The overall aim of the research described in this thesis was to understand the effects of climate change on northern peatland soil nitrogen cycling, and to illuminate the mechanistic connections between the biological processes underlying these effects. More specifically we address the following research questions:

1. To what extent does climate change affect soil nitrogen dynamics in northern peatlands?
2. What is the relative importance of direct (changes to temperature and moisture) and indirect (alterations to patterns of substrate input into the soil) effects of climate change in determining changes in peatland nitrogen cycling under climate change?
3. Are changes in peatland nitrogen cycling upon climate change linked to concomitant changes in the potential activity of soil enzymes and/or the composition of the soil microbial community?

1.5 Approach

My approach was to use a combination of field and laboratory experiments targeting the effects of climate change on different levels of organization in the peatland soil system: measures of nitrogen flux rates (with a focus on depolymerization of organic nitrogen); quantification of potential soil enzyme activities; and molecular characterization of the soil microbial community composition. This multi-level approach allows for the refinement of our mechanistic understanding — going beyond the traditional black-box approach to soil biogeochemistry that measures the stocks
and flows of various elemental forms, without considering the biological processes which control the various transformations. For the field experiments I took advantage of a long-term climate manipulation experiment in an ombrotrophic Sphagnum peat bog near Abisko, northern Sweden. This experiment, running since 2000, incorporates a factorial design manipulating air and soil temperature in spring and summer, and snow accumulation during winter (Dorrepaal et al., 2004), and has the added advantage that extensive data on the response of vegetation and soil respiration has been collected in parallel to the studies described in this thesis (Aerts et al., 2006b; Dorrepaal et al., 2006; Aerts et al., 2007; Keuper et al., 2011). This allows the placing of the results into the broader context of changes in other components of the ecosystem.

1.6 Outline of the thesis

The next five chapters of this thesis describe different approaches to the general question of describing and understanding the response of the nitrogen cycle in a northern peatland to climate change.

Chapter 2, presents a brief review of the literature related to organic nitrogen cycling in alpine and (sub-) arctic environments and relates it to studies concerning the activities of soil enzymes. This review explores some of the key issues involved in trying to make a functional link between soil biogeochemical cycles and enzyme measurements. We identify a gap in the literature, in that few investigators have attempted to explicitly link soil organic N fluxes with measurements of the enzymes that carry out the relevant transformations.

Chapters 3 and 4 describe an initial attempt at filling this gap, involving measurements of nitrogen pool dynamics, soil enzyme activities and microbial community structure taken in the long-term climate change experiment introduced above. Chapter 3 explores the effects of experimental climate manipulation on each of the three levels of organization mentioned above. Chapter 4 focuses on the effects of the treatments on soil enzyme activity, and in particular, the degree of coupling between C and N cycle enzymes over the summer growing season, an important issue when attempting to use enzyme measurements to understand soil biogeochemical cycling.

Chapter 5 presents the results of experiments involving laboratory incubations of peatland soils to explore a hypothesis about the greater importance of indirect warming effects on soil processes that arises in the Discussion of Chapter 3. More specifically, we aimed to separate warming effects on carbon and nitrogen fluxes, enzyme
activities and microbial community composition into direct effects due to temperature alone, and indirect effects through changes to substrate supply.

Chapter 6 gives the results of an experiment that employed high-throughput sequencing to profile bacterial community and asked whether the effects of climate change on peatland C and N cycles are reflected in the phylogenetic community composition of the microbes that are putatively responsible for them.

Chapter 7 provides a synthesis of the results from the separate studies to address the main research questions and discuss the implications for our understanding of climate change effects on soil processes and the choice of methodologies for understanding these effects. An updated version of Figure 1-2 is presented, highlighting the processes and feedbacks which were identified as critical in driving climate change effects on peatland N cycles.