A clash of plants

Vegetation succession and its interaction with

permafrost dynamics in the Arctic lowland tundra

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Contents

Chapter 1: General introduction

1.1 Climate warming and increasing precipitation in the Arctic

The high-latitude regions have experienced significant changes during the last few decades due to global warming (ACIA, 2005; IPCC, 2014). Compared to other regions of the world, the Arctic areas are more vulnerable to the changing climate (Hinzman et al., 2005; McGuire et al., 2006). The increase in temperature in the Arctic is 0.2 to 0.4 °C per decade, almost double of the global mean since the beginning of the 20th century (Chapin et al., 2000; Keyser et al., 2000; Serreze et al., 2000). The air temperature in the Russian Arctic has increased continuously between 1965 and 2005 (Fedorov et al., 2014). Similar temperature increases have been observed in the Arctic parts of North America (Osterkamp and Romanovsky, 1999; Osterkamp, 2005) and Europe (Harris et al., 2009). This trend is expected to continue in the near future (IPCC, 2014). Similarly, the Arctic experienced a significant increase in precipitation over the 20th century (Bintanja and Selten, 2014), while the precipitation is expected to increase further by more than 50 percent before the end of the 21st century (Kattsov et al., 2009).

1.2 Permafrost degradation in the Arctic tundra

Permafrost (soil or rock that remains at or below 0 °C for 2 years or more) covers approximately 22% of the northern hemisphere (Zhang et al.). The stability of permafrost is crucial to the global climate, as greenhouse gas release from degrading permafrost soils may amplify climate warming (Schuur et al., 2015). The continuous permafrost in the Arctic zone has been considered to be stable (Jorgenson et al., 2001), but the risk of local permafrost degradation in this region is substantial due to the high ice content in the top layer of the permafrost (Nelson et al., 2001). The studies of Ping et al. (2013) and Iwahana et al. (2014) showed that the ice content of the permafrost underlying Arctic tundra is around 75%-80% by volume. In Alaska around 10-30% of the Arctic landscapes with continuous permafrost in Alaska has experienced permafrost degradation due to the influence of modest climate change since the 1940s (Jorgenson et al., 2006).

Approximately one third to half of the world soil carbon is stored in the permafrost at the high latitudes (Mack et al., 2004; Tarnocai et al., 2009; Hugelius et al., 2014; Schuur et al., 2015). Climate warming might transfer the Arctic tundra from a carbon sink to a carbon source, due to enhanced respiration rates (Koven et al., 2011). Some other studies drew an opposite conclusion, as warming would increase net carbon sequestration due to lengthening of the growing season (Qian et al., 2010), showing that the influence of climate warming on the carbon balance in the Arctic tundra is uncertain. The study by Mcguire et al. (2012) suggests large temporal and spatial variability in greenhouse gas fluxes in the Arctic tundra, based on the analysis of observational data and simulations using process-based models and inversion models. Therefore, it is still uncertain whether the Arctic tundra is at present a net carbon sink or a carbon source.

The thawing of permafrost has accelerated in recent decades (Serreze et al., 2000). Most researchers focus on gradual top-down melting of permafrost, but also abrupt small-scale permafrost thaw (thermokarst) became more widespread in the Arctic zones with ice-rich

continuous permafrost (Schuur et al., 2015). Climate change and disturbance are considered to be the major factors that trigger the abrupt small-scale permafrost thawing (Jorgenson et al., 2006; Raynolds et al., 2014). The melt of ground ice, which is richly stored in the permafrost, results ultimately in sudden permafrost collapse. The spectral analyses and field observations by Jorgenson et al. (2006) showed that the number of thermokarst pits has rapidly increased in both uplands and lowlands in Alaska since 1940s, while the extent of degradation at different sites was largely related to the changes in local air temperature.

1.3 Vegetation changes in Arctic tundra

Being the northern-most biome subject to the strongest climatic changes, the Arctic tundra is facing rapid vegetation shifts driven by climate warming. Pearson et al. (2013) predicted that at least half of the vegetated area in the Arctic will experience significant vegetation shifts in the next 40 years. Their simulations suggest that the woody cover will increase by as much as 52% (Pearson et al., 2013). Shrub expansion in Arctic low-shrub tundra has been widely observed at many sites throughout Alaska (Sturm et al., 2001), Canada (Hudson and Henry, 2009) and other Arctic tundra regions (Myneni et al., 1997; Myers-Smith et al., 2011; Frost and Epstein, 2014). Experimental warming studies suggested that both cover and biomass of deciduous shrubs increased in response to higher soil nutrient availability caused by the warming treatments in these experiments (Wahren et al., 2005). In several fertilization studies in Arctic tundra, the accumulation of biomass of both deciduous shrubs and graminoids were promoted, while the evergreen shrub, lichen and moss biomass declined (Mack et al., 2004; Bret-Harte et al., 2008). These results suggest that increased nutrient availability in Arctic tundra due to warmer temperatures leads to dominance of deciduous shrubs.

However, abrupt small-scale permafrost degradation caused by warming influence vegetation composition in a different way (Jorgenson et al., 2001; Jorgenson et al., 2013). Abrupt permafrost degradation in the Arctic ecosystems can lead to significant soil surface depression (Nelson et al., 2001), impounding water in the subsiding areas (Jorgenson et al., 2013) and result in thicker active layers (the seasonally thawed soil on the top of the permafrost). The depressions trap more snow in winter, which leads to increased insulation during winter and further warming and thawing of the permafrost (Osterkamp et al., 2009). The resulting change in soil moisture leads to a dramatic shift of the local vegetation composition (Jorgenson et al., 2006). As the abrupt small-scale permafrost thawing rapidly raises the local water table (Yoshikawa and Hinzman, 2003), the original shrubs, lichens and tussock forming sedges are rapidly replaced by hydrophilic sedges and *Sphagnum* mosses (Shur and Jorgenson, 2007; Osterkamp et al., 2009). If such replacement of shrubs by sedges would occur more frequently in a warmer climate, it would constrain the currently observed shrub expansion in the Arctic.

Moreover, as permafrost dynamics in Arctic tundra interacts continuously with vegetation succession (Osterkamp et al., 2009), the vegetation changes of Arctic tundra have important impacts on permafrost stability. Shur and Jorgenson (2007) stated that although the stability of ice-rich permafrost in Arctic tundra is mainly influenced by climate, vegetation processes have important impacts on permafrost stability as well. The shifts in vegetation composition can significantly affect the absorption of shortwave solar radiation, thereby influencing summer

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surface albedo, soil shading and local atmospheric heating (Chapin et al., 2005; Juszak et al., 2016). Vegetation composition also affects soil surface roughness, snow cover, soil moisture and heat conduction near the soil surface (Sturm et al., 2005; Loranty et al., 2011). However, the influence of vegetation on permafrost stability is rather complicated. For instance the study by Juszak et al. (2014) showed that dense shrub cover provides soil shading, which mitigates the warming effects of increasing air temperature. Blok et al. (2010) drew a similar conclusion based on their multiple-year field observation. However, this statement is challenged by Tape et al. (2006), who suggested that the current shrub expansion may lead to higher soil temperatures in the future, as particularly taller shrubs have lower albedo and absorb more solar radiation and result in thicker insulating snow cover in winter.

Moreover, the changes in vegetation composition of Arctic tundra can influence the greenhouse gas emission from soil. For instance, *Eriophorum* spp. sedges can quickly transport methane, one of the major greenhouse gases, from water-saturated anaerobic parts of the soil to the air through its internal aerenchyma structure (Ström et al., 2005). Oppositely, entophytic methane oxidizing bacteria in submerged *Sphagnum* mosses can significantly suppress methane emissions (Parmentier et al., 2011). Therefore, vegetation shifts may have important impacts on the greenhouse gas balance of the Arctic tundra ecosystem.

Understanding the vegetation succession after small-scale permafrost degradation is desperately needed to predict the changes of the Arctic tundra ecosystem in the future. Jorgenson et al. (2006) suggested that vegetation succession provides a strong negative feedback that limits the vertical extent of the abrupt small-scale permafrost degradation, which strong stabilizes the permafrost. Rapid peat accumulation in thermokarst pits facilitates the recovery of the degraded permafrost (Osterkamp et al., 2009). With the vegetation succession, the degraded permafrost may experience a cyclic degradation-recovery process (Jorgenson et al., 2006). However, few studies revealed the complete vegetation succession route in Arctic tundra with abundant small-scale permafrost degradation yet.

1.4 Study area

For a better understanding of the dynamics of tundra shrub vegetation and the vegetation succession in response to small-scale permafrost degradation in Arctic tundra, I have conducted a four-year study at a Northeastern Siberian tundra site since 2011. The research station is located in a former thermokarst lake bed in Kytalyk Nature Reserve, Russian Federation (70.83°N, 147.49°E, Fig. 1.1). The study site is located in an understudied area of Arctic tundra and can be classified as highly vulnerable to climate change due to the ice-rich permafrost and yedoma soils, which are known for their high content of frozen soil organic carbon. Small-scale permafrost degradation has been widely observed at the study site, which provided a good opportunity to investigate the complicated interactions between ecological processes and permafrost dynamics.

Fig. 1.1 *Location of the Chokurdakh Scientific Tundra Station, Kytalyk Nature Reserve in Northeastern Siberia.*

The dominant plant type at our study site consists of different dwarf shrub species (e.g. *Betula nana* L., *Vaccinium vitis-idaea* L., *Vaccinium uliginosum* L., *Rhododendron tomentosum* Harmaja and *Salix pulchra* Ch.). This category of plant species occupies 35% of the whole Arctic tundra zone (Walker, 2000). The other abundant plant types are graminoids (e.g. *Eriophorum angustifolium* Honck and *Eriophorum vaginatum* L.) and *Sphagnum* mosses (e.g. *Sphagnum fuscum* (Schimp.) H. Klinggr., *Sphagnum pulchrum* (Lindb.) Warnst., *Sphagnum flexuosum* Dozy and Molk. and *Sphagnum squarrosum* Crome). The shrubs, especially *B. nana*, dominate the slightly elevated positions with well-drained soil, while sedges such as *E. angustifolium* occupy drainages and other areas with water-saturated soil. Meanwhile, *Sphagnum* mosses widely occur in the transition zones. The warmest Arctic tundra zone has mean July temperatures of 9 to 12 °C, and its south parts are often adjacent to boreal forests (Walker, 2000). The tundra-taiga transition can be found at approximately 40 km from our study site (Liang et al., 2014).

1.5 Aim and objectives of the thesis

Observations of drowning shrubs in small-scale permafrost collapses, invading sedges and expanding *Sphagnum* moss carpets at our study site have led to the hypothesis that vegetation succession in these ecosystems could be cyclic and closely related to permafrost dynamics. Abrupt permafrost degradation, which can be triggered by reduction of the deciduous shrub cover (Nauta et al., 2015), results in local soil subsidence and increasing soil moisture in the newly formed depressions. The new water-saturated environment favors the growth of sedges, whereas the dwarf shrubs do not tolerate the anoxic conditions and die. *Sphagnum* mosses could gradually invade the established sedge dominated patches, and eventually create proper substrates for the establishment and growth of the deciduous shrubs.

In this thesis I tried to answer the following questions:

1. How do *B. nana* shrubs establish, grow and reproduce in the tundra in Northeastern Siberia?

2. How does the vegetation composition shift after the degradation of *B. nana* shrub patches?

3. Is vegetation succession in these communities strongly connected with hydrological and permafrost dynamics?

4. What impacts have *Sphagnum* mosses on the vegetation succession in the tundra?

To provide answers to these questions, I combined multiple research methods, which include field experiments and observations, dendrochronological analyses and molecular, microsatellite methods (Fig. 1.2). First of all, I explored the effects of climate factors, eqspecially precipitation and temperature, on the germination and establishment of *B. nana* shrubs. I also investigated which reproduction mode of *B. nana* shrubs, vegetative reproduction (clonal ramets) or seed dispersal, is predominant in tundra. The vegetation development after the removal of *B. nana*, which resulted in soil subsidence, was monitored and I explored the relationships between vegetation shift and local permafrost degradation. At last but not the least, I studied the invasion of *Sphagnum* mosses in the sedge dominated areas like drainages and ponds, and its influence on the soil conditions which may enable shrub establishment.
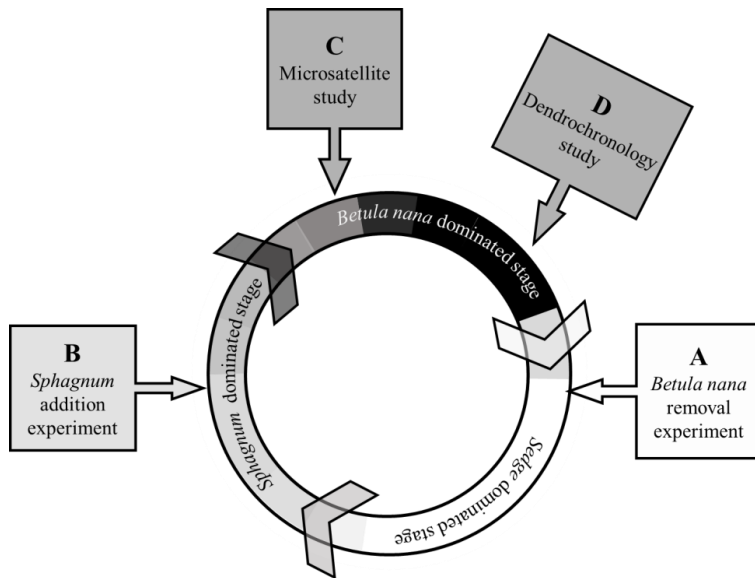
Fig. 1.2 *The hypothesized vegetation succession cycles in the tundra of Northeastern Siberia with the position of the four studies (A, Betula nana removal experiment; B, Sphagnum addition experiment; C, Microsatellite study; D, Dendrochronology study) which we performed Different colors represent different soil moisture levels (lighter color, wetter soil condition; darker color, drier soil condition)*

In chapter 2, I investigated how vegetation and soil conditions shifted after the removal of *B. nana* shrubs in the original *B. nana* dominated patches. I followed a nine-year *B. nana* removal experiment which was initiated by Blok et al. (2010) in 2007. The vegetation changes in this experiment during nine years were analyzed to check whether the *B. nana* removal plots had changed toward the sedge dominated status as we observed in thaw ponds that had resulted from abrupt permafrost thaw outside the experiment. I monitored the development of permafrost degradation after the removal of *B. nana*, which had been measured in 2012 by Nauta et al. (2015).

I also studied the *Sphagnum* dominated stage, the stage before the *B. nana* dominated stage (Fig. 1.2). Chapter 3 describes a four-year *Sphagnum* addition experiment in which I assessed the influence of newly formed *Sphagnum* carpets in the sedge dominated area on soil conditions, vegetation composition and greenhouse gas fluxes. I hypothesized that the new *Sphagnum* moss carpet significantly changes local soil conditions, creates suitable environments for the germination of deciduous shrubs like *B. nana* and limits the growth of sedges.

In Chapter 4 and Chapter 5, two different techniques, dendrochronological measurements and the microsatellite (DNA marker) method, were applied. In these two chapters I tried to understand how shrubs establish and become dominant. The dendrochronology study helped us to reveal whether climate factors affected the germination and the establishment of the *B. nana* shrubs. The microsatellite study then facilitated us to explore the prevalent reproduction mode of the *B. nana* shrubs. Consequently, I obtained a complete picture of the history of the present *B. nana* dominated patches in the tundra of the Northeastern Siberia.

Finally, Chapter 6 gives an overview of the most important results and puts them in the broader context of Arctic ecology. I furthermore made suggestions for future researches on the Arctic tundra ecosystems in the context of climate change.

Chapter 2: Thaw pond development and initial vegetation succession in experimental plots at a Siberian lowland tundra site

Bingxi Li , Monique Heijmans, Daan Blok, Peng Wang, Sergey Karsanaev, Trofim Maximov, Jacobus van Huissteden, Frank Berendse

2.1 Abstract

Permafrost degradation has the potential to change the Arctic tundra landscape. We observed rapid local thawing of ice-rich permafrost resulting in thaw pond formation, which was triggered by removal of the shrub cover in a field experiment. This study aimed to examine the rate of permafrost thaw and the initial vegetation succession after the permafrost collapse. In the experiment, we measured changes in soil thawing depth, plant species cover and soil subsidence over nine years (2007-2015). After the abrupt thaw, the soil subsidence in the removal plots continued indicating further thawing of permafrost albeit at a much slower pace: 1 cm yr^{-1} over 2012-2015 vs. 5 cm yr^{-1} over 2007-2012. Grass cover strongly increased after the initial shrub removal, but later declined with ponding of water in the subsiding removal plots. Sedges established and expanded in the wetter removal plots. Thereby, the removal plots have become increasingly similar to nearby 'natural' thaw ponds. The nine years of field observations in a unique shrub removal experiment at a Siberian tundra site document the trajectories of small-scale permafrost collapse and the initial stage of vegetation recovery, which is essential knowledge for assessing future tundra landscape changes.

Key words: Arctic tundra; *Betula nana*; permafrost degradation; thermokarst; vegetation dynamics

2.2 Introduction

The Arctic has experienced a continuous period of warming during the last three decades and it is predicted that this trend will continue in the near future (IPCC 2013). The warming has triggered a series of marked changes in the Arctic tundra ecosystems, including permafrost degradation (Hinzman et al. 2005; Jorgenson et al. 2015), acceleration of greenhouse gas release (IPCC 2013; Tarnocai et al. 2009) and shifts in vegetation composition (Chapin and Shaver 1996; Elmendorf et al. 2012; Fraser et al. 2014; Jia et al. 2009; Tape et al. 2006). One of the most striking changes in vegetation composition is the extensive shrub expansion that has been observed in many parts of the Arctic (Myers-Smith et al. 2011). Along the Arctic tree line, tree and shrub expansion seem to be concentrated in upland tundra landscapes and less in lowland tundra (Frost and Epstein 2014).

Occurrence of abrupt permafrost thaw and increasing numbers of thaw ponds or thermokarst pits have been reported for Alaskan and Canadian Arctic tundra sites (Beck et al. 2015; Jorgenson and Grosse 2016; Jorgenson et al. 2010; Schuur et al. 2015). At our study site in Siberian lowland tundra, thaw ponds occur as well and their number has doubled between the 1970s and 2010 based on a comparison of satellite images (unpublished data). The ice content in the top layer of the permafrost underlying this region can reach as high as 80% by volume (Iwahana et al. 2014; Kanevskiy et al. 2013), making the permafrost highly sensitive to climate warming. The melting of ice in the top layer of permafrost can lead to soil subsidence and ponding of water. The depressions that evolve from the soil subsidence effectively trap snow and water which contribute to further thawing and thaw pond formation (Nauta et al. 2015). Little is known about what triggered the local abrupt thaw, how these thaw ponds have developed over time and how vegetation succession and recovery eventually takes place.

We set up a *Betula nana* L. shrub removal experiment at a northeast Siberian tundra site, to

examine the impact of vegetation change on seasonal thawing of permafrost (Blok et al. 2010). In five 10-meter diameter *B. nana* dominated plots, the aboveground parts of the *B. nana* dwarf shrubs had been removed in 2007. Local permafrost collapse and soil subsidence had been observed by the 5th year in the removal treatment, resulting in ponding of water that shifted the plots from a net sink into a source of methane (Nauta et al. 2015).

We continued the measurements and analyzed all data collected between 2007 and 2015, to have a complete picture of the abiotic and biotic changes in the *B. nana* removal and control plots. We also compared the vegetation change in the *B. nana* removal plots with that in the natural thaw ponds at the same study site.

The goal of this study was to gain insight in thaw pond development and the initial stage of vegetation succession after abrupt permafrost thaw by examining the changes over 9 years in thawing depth, soil subsidence and vegetation composition in the *B. nana* removal experiment. We hypothesized that after 2012 1) the soil subsidence continued in the *B. nana* removal plots, 2) the vegetation composition shifted further, 3) and became more similar to the vegetation of the natural thaw ponds occurring adjacent to the experimental plots.

2.3 Materials and methods

2.3.1 Site description

The research site is part of the Chokurdakh Scientific Tundra Station, located in the Kytalyk Nature Reserve in the lowlands of the Indigirka River, Sakha Republic, Russian Federation (70°49'N, 147°28'E). The study area consists of poorly-drained lowland tundra and is characterized by thick continuous permafrost with a shallow active layer on top. The vegetation of the research area is classified as tussock-sedge tundra, dominated by *Eriophorum* spp. sedges and deciduous shrubs like *B. nana* and moss (G4), in the Circumpolar Arctic Vegetation Map (Walker et al. 2005). *B. nana* is dominant on slightly elevated shrub patches, while *Sphagnum* mosses and *Eriophorum* sedges are the most common plant types in waterlogged depressions surrounding the shrub patches and in thaw ponds. The research site is in the Low Arctic climate zone with Chokurdakh (WMO station 21946) mean annual air temperature of −13.4 °C (1981-2010) and mean annual precipitation of 196 mm (1981-2010). An extensive site description can be found in Nauta et al. (2015).

2.3.2 Removal experiment and natural thaw ponds

The *Betula* removal experiment was set up in the summer of 2007. For this experiment, 10 dwarf-shrub plots (5 pairs) were selected in the former bed of a thermokarst lake. In each plot pair, one was the control plot and the other the removal plot. *B. nana* was the dominant species in all plots. In July 2007 the aboveground biomass of *B. nana* in the 10-m diameter removal plots was clipped manually by using branch clippers. Resprouted *B. nana* shoots (from belowground coarse roots) were clipped again in the summer of 2010 and 2013. In 2013, we selected four 'natural' thaw ponds with similar size in the same drained thaw lake basin, for comparison with the subsided *B. nana* removal plots. The word "natural" is used here to indicate the contrast with the developing thaw ponds in the experiment. We do not know the mechanism that triggered the abrupt permafrost thaw in the studied thaw ponds, but we presume that human-induced vegetation disturbance was

unlikely.

2.3.3 Vegetation composition

The point quadrat method was used to measure the vegetation composition of each plot annually in mid-July. We assessed the cover of vascular plant species and moss and lichen groups of each plot by recording the presence at 169 evenly distributed points (13 × 13 matrix) covering 10 × 10 m. We only used the records inside the 10-m diameter plot areas in the following analyses. We vertically lowered a thin metal pin and recorded all the plant species that the pin touched while it was brought down at each point, with a maximum of one hit per species per point. The cover of a plant type was defined as the number of times this plant type was recorded per plot divided by the total number of points within the 10-m diameter plot. Therefore, the value of a plant species cover was between 0% and 100%. The same method was also used for measuring the vegetation composition of the natural thaw ponds in mid-July of 2013. We measured the vegetation composition before the *B. nana* removal treatment in the removal plots in 2007 and 2010 but after the treatment in 2013.

To illustrate the changes in vegetation composition over time, we made mosaic like vegetation maps using "ggplot2", "gird", "Rcolorbrewer" and "scale" packages from the R Project for Statistical Computing. The top species hit at each point from 2007, 2009, 2011, 2013 and 2015 was used for the vegetation maps. We categorized the plant species into 14 groups with unique colors (*B. nana*, Salix spp., other shrub, grass, sedge, forbs, lichen, non-*Sphagnum* moss, *Sphagnum*, water/mud (bare soil) surface, shrub litter, grass litter, sedge litter). The "other shrub" group included *Rhododendron tomentosum* Harmaja, *Rubus chamaemorus* L., *Vaccinium uliginosum* L., and *Vaccinium vitis-idaea* L.. The main grass species was *Arctagrostis latifolia* (R.Br.) Griseb. The "sedge" group included *Eriophorum angustifolium* Honck., *Eriophorum vaginatum* L., and *Carex aquatilis* var. minor Boott.

We compared the cover of each plant group in the control and the removal plots over the whole time series and annually using Repeated Measures ANOVA and one-way ANOVA tests, respectively, in SPSS software (IBM SPSS Statistics for Windows, ver. 22.0; IBM Corp., Armonk, NY, USA). The normal distribution of each variable was examined before this test and the tests mentioned later. All data series whose residuals were not normally distributed were log-transformed and their residuals were normalized.

To visualize the vegetation shift in the two plot types between 2007 and 2015 and the similarity with the natural thaw ponds, a PCA figure was made by using CANOCO (canonical community ordination) software (version 4.5, Microcomputer Power, NY, USA). The same vegetation groups used in the vegetation maps were also implemented here. We calculated the average cover of each plant group in the control and the removal plots for each year and loaded the results in CANOCO for the final PCA figure. We included the four natural thaw ponds (measured in 2013) in the PCA figure for comparison with the removal plots. Finally, we made a dendrogram figure to explore the similarity in vegetation composition of the individual plots using SPSS software. The point quadrat data measured in 2013 were used in this test.

2.3.4 Thaw depth and surface elevation

The soil active layer thickness (ALT) of each plot in the *B. nana* removal experiment has been measured in late July of each year. ALT was measured from the top of the moss/litter/bare-soil surface to the top of the permafrost at nine points within each plot using a bluntly tipped steel probe. We compared the ALT of the control plots and the removal plots using RM-ANOVA with year as repeated factor within plots. ALT differences between the control plots and the removal plots in specific years were analyzed using one-way ANOVA tests.

The relative surface elevation of each plot was measured in 2015 using an optical levelling instrument and compared to the measurements in 2012 (Nauta et al. 2015). Two diagonal transects perpendicular to each other, extending several meters beyond the plot borders, were introduced to measure the relative surface elevation in each plot. We followed the same procedures and steps that were made in the preceding years, guaranteeing that the data series were comparable, although the measurements had not been done at exactly the same spots within the transects. Each transect had 19 evenly distributed spots every 1 m for the elevation measurement within the 10-m diameter plot, and at least 8 extra spots outside the plot area (4 spots per side). To calculate the relative elevation, the mean elevation of the 16 spots of the two transects just outside the plot was considered as the 0 m reference, assuming the elevation of these spots had changed little between 2012 and 2015. In this way, we transformed the original elevation data into the relative elevation data series that could be used to compare the treatments. The mean relative elevation of each plot type in 2012 and 2015 were compared by using linear mixed model tests. In the test, plot was treated as the subject, with year and distance to plot center repeated within plot, and treatment (control, removal), year (2012, 2015) and distance to plot center (0 to 5 m) were the fixed factors. RM-ANOVA tests were furthermore used for comparing the elevations of each plot in 2012 and 2015.

2.4 Results

2.4.1 Abiotic changes in the Betula nana removal experiment

The soil subsidence in the removal plots continued after 2012, resulting in further thaw pond development (Fig. 2.1). Surface elevation changes between 2012 and 2015 depended on the removal treatment (treatment × year interaction: $F = 4.68$, $p = 0.033$). The removal plots had experienced significant elevation changes between 2012 and 2015 (control plots: $F = 26.20$, $p = 0.081$; removal plots: $F = 13. 10$, $p = 0.001$, Fig. 2.2, Fig. 2.A1). The subsidence between 2012 and 2015 of the removal plots was significantly larger than that of the control plots (removal plots: 7.6 ± 1.3 cm; control plots: 2.3 ± 0.6 cm; $F = 26.35$, $p < 0.001$). In the removal plots, distance from plot center significantly affected the elevation changes (control plots: $F = 3.20$, $p = 0.844$; removal plots: $F = 4.31$, $p = 0.011$). The subsidence progressively increased from the margins to the centers of the removal plots (slope = 1.5 cm m^{-1}, $R^2 = 0.93$).
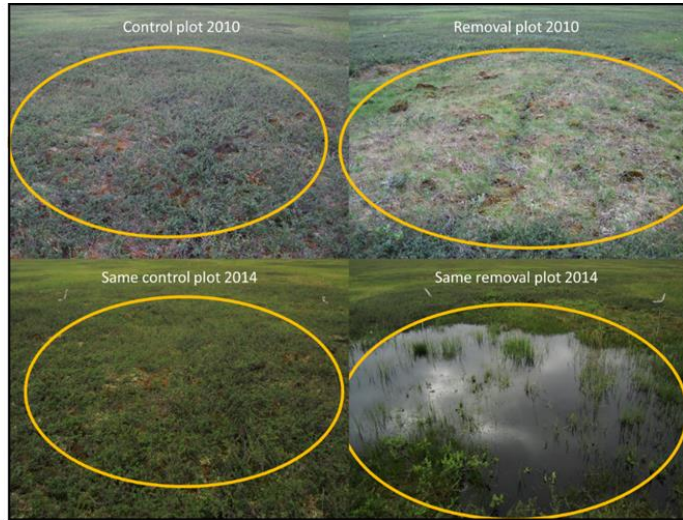
Fig. 2.1 *One pair of a control plot with dense Betula nana shrub vegetation (left) and a 10-m diameter removal plot in which the aboveground biomass of B. nana had been removed (right). Photos were taken from the same position in 2010 and 2014. The control plot has not changed, whereas this removal plot has turned into a thaw pond. The yellow circle indicates the area of the 10-m diameter plot.*
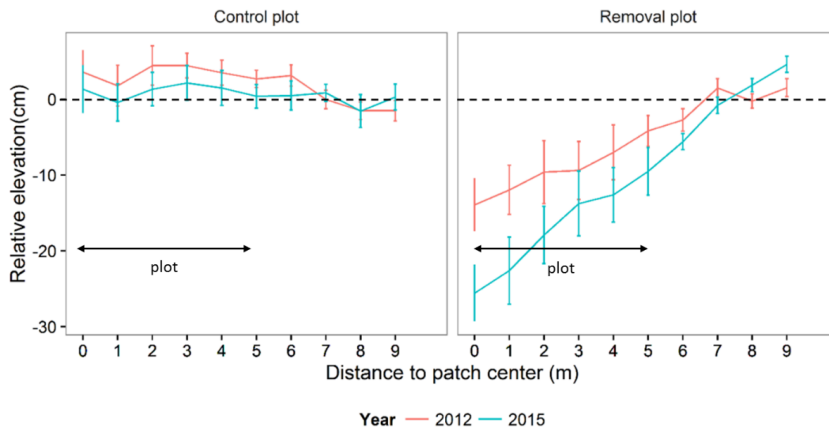
Fig. 2.2 *The relative surface elevation in 2012 (red lines) and 2015 (blue lines) of the control plots (left panel) and the B. nana shrub removal plots (right panel) at increasing distance from the plot center. Data are mean values ± s.e.m., n = 5 plots. The average elevation at 6-9 m from the plot center (n = 16 spots) was taken as the zero reference for each plot.*

In all eight years after the initial removal of *B. nana* cover, the late-July thawing depth (ALT) in the

removal plots was significantly deeper than in the control plots ($F = 105.92$, $p < 0.001$ in 2015, Fig. 2.3). The ALT in the removal plots increased over years while the ALT in the control plots was rather stable (interaction between year and treatment: $F = 17.48$, $p < 0.001$). ALT in the removal plots had continuously increased from 2009 to 2012 (slope = 5.6 cm y^{-1}, R^2 = 0.95) but became stable afterwards (slope = 0.26 cm y^{-1}, R^2 = 0.01). Similarly, the difference in ALT between control and removal plots did not increase further after 2012 (Fig. 2.3).
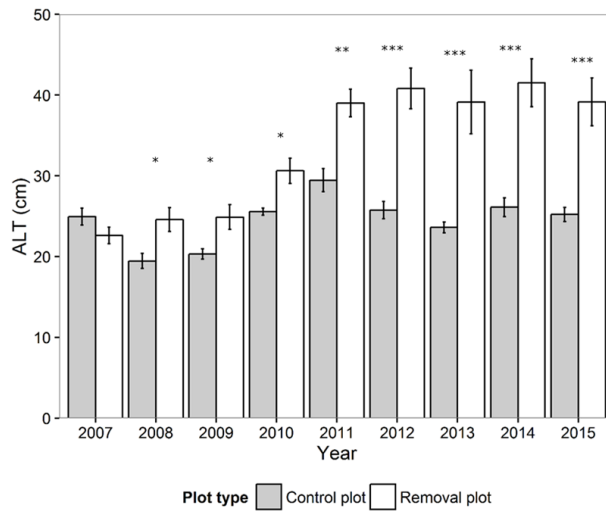
Fig. 2.3 *Late-July active layer thickness (ALT) in the B. nana shrub removal plots (Removal) and control plots (Control) from the start of the experiment (2007) until 2015. Data are mean values ± s.e.m., n = 5 plots. *, ** and *** indicate significant differences between the two treatments (p < 0.05, p < 0.01, p < 0.001, respectively).*

As a result of the soil subsidence and increased ALT in the removal plots relative to the control plots, the permafrost table lowered on average by 30 cm over 8 years in the removal plots (Fig. 2.4). However, after 2012 the permafrost thawing rate strongly declined from 5.4 cm y^{-1} over 2007-2012 to 1.0 cm y^{-1} over 2012-2015.
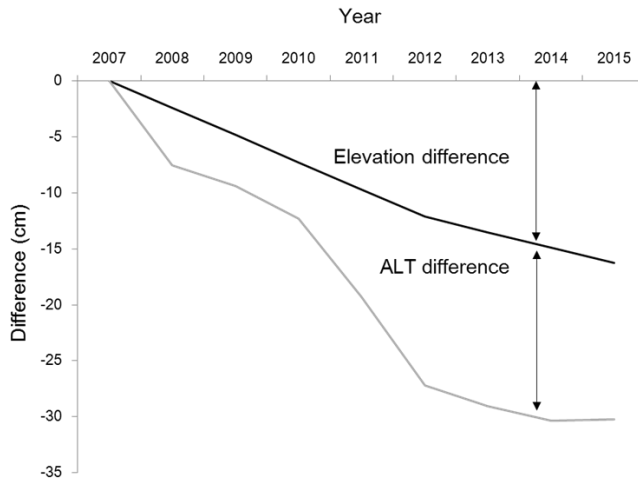
Fig. 2.4 *Soil subsidence and the thawing of permafrost in the B. nana shrub removal plots (2007-2015). The black line indicates the lowering of the soil surface (soil subsidence), based on relative elevation measurements in 2012 and 2015. The grey line indicates the lowering of the permafrost table, calculated as the sum of soil subsidence and the increase in thawing depth (ALT, measured relative to the soil surface) due to the removal treatment.*

2.4.2 Vegetation shifts in the Betula nana removal experiment

In 2007, the initial covers of all major plant groups in the control plot and the removal plots were not significantly different (all *p* > 0.05, Fig. 2.5). While the covers of the major plant types in the control plots were rather stable, the cover of *B. nana*, grass, sedge and mud/water in the removal plots showed large changes (Fig. 2.5). In the removal plots most vegetation change over the whole experiment period can be attributed to the changes in the covers of the two main plant types: *B. nana* shrubs, whose aboveground biomass had been removed in 2007, 2010 and 2013, and grasses. The cover changes of both plant types were over 30%. The clipping of *B. nana* dwarf shrubs drastically reduced its cover in the removal plots, as intended, from 78% ± 6% in 2007 to 13% ± 1% in 2015 (Fig. 2.5). During this process, the regrowth of *B. nana* in the removal plots was substantial in 2009 and 2010 but declined again after the removal of regrowth (Fig. 2.5). The grass cover strongly increased from 2009, reaching a peak in 2011 (55% ± 6%), but declined thereafter and dropped back to 23% ± 6% in 2015.
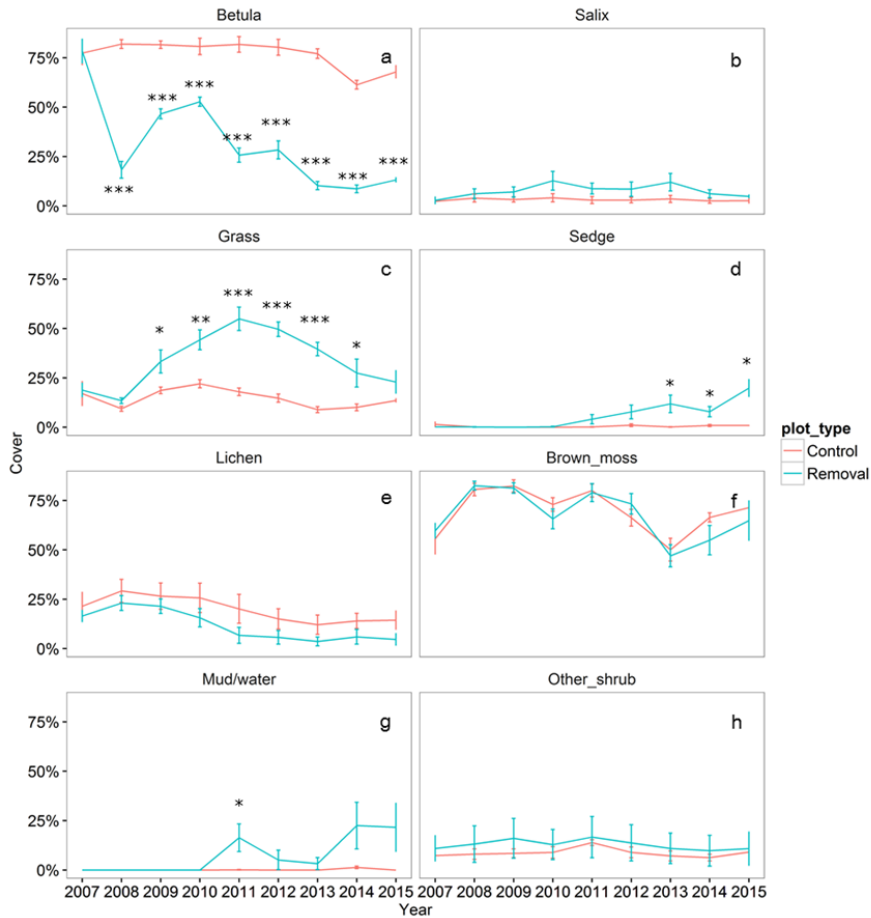
Fig. 2.5 *The cover (%) of seven plant groups and exposed mud/water in the B. nana shrub removal experiment between 2007 and 2015. Data are mean values ± s.e.m., n = 5 plots. The blue lines indicate the cover in the B. nana removal plots and the red lines are the values in the control plots. *, ** and *** indicate significant differences between the two treatments (p < 0.05, p < 0.01, p < 0.001, respectively). Aboveground biomass of B. nana had been clipped off in 2007, 2010 and 2013. In 2007 and 2010 the vegetation measurements took place before the removal, in 2013 the vegetation survey was made after the removal of regrowth.*

The removal of *B. nana* also changed the cover of sedges and mud/water significantly. Both mud/water and sedges were present in the removal plots since 2011, and reached around 20% cover in 2015 (mud/water: 22% ± 12%, sedge: 20% ± 5%). In the very wet summer of 2011, the cover of mud and standing water abruptly increased from 0% to 16% and 7%, but fell back to around 5% in the drier summers of 2012 and 2013 (Fig. 2.5).

The mosaic-like vegetation maps show the changes in species composition in the canopy of the five removal plots over the years (Fig. 2.6). In all five removal plots the canopy was dominated by

B. nana shrubs before the *B. nana* removal took place in the removal plots. With the decrease of *B. nana*, the grasses strongly increased in cover, becoming the most common plant type, with a cover of at least 34%, in all five removal plots in 2011 (Fig. 2.6). Sedges first appeared along the margin of removal plot 3 in 2009 and became the most common plant group in the canopy in removal plot 2 (23%) and 3 (31%) in 2015, while the sedge cover in the other three plots varied between 10% and 15% (Fig. 2.6). Moreover, 41% of the area of removal plot 5 was bare mud or water in 2015. In the natural thaw ponds, sedge was the dominant plant type, accompanied by some *Sphagnum* moss in pond 3 and 4, while *B. nana* was dominant just outside the pond (Fig. 2.6).
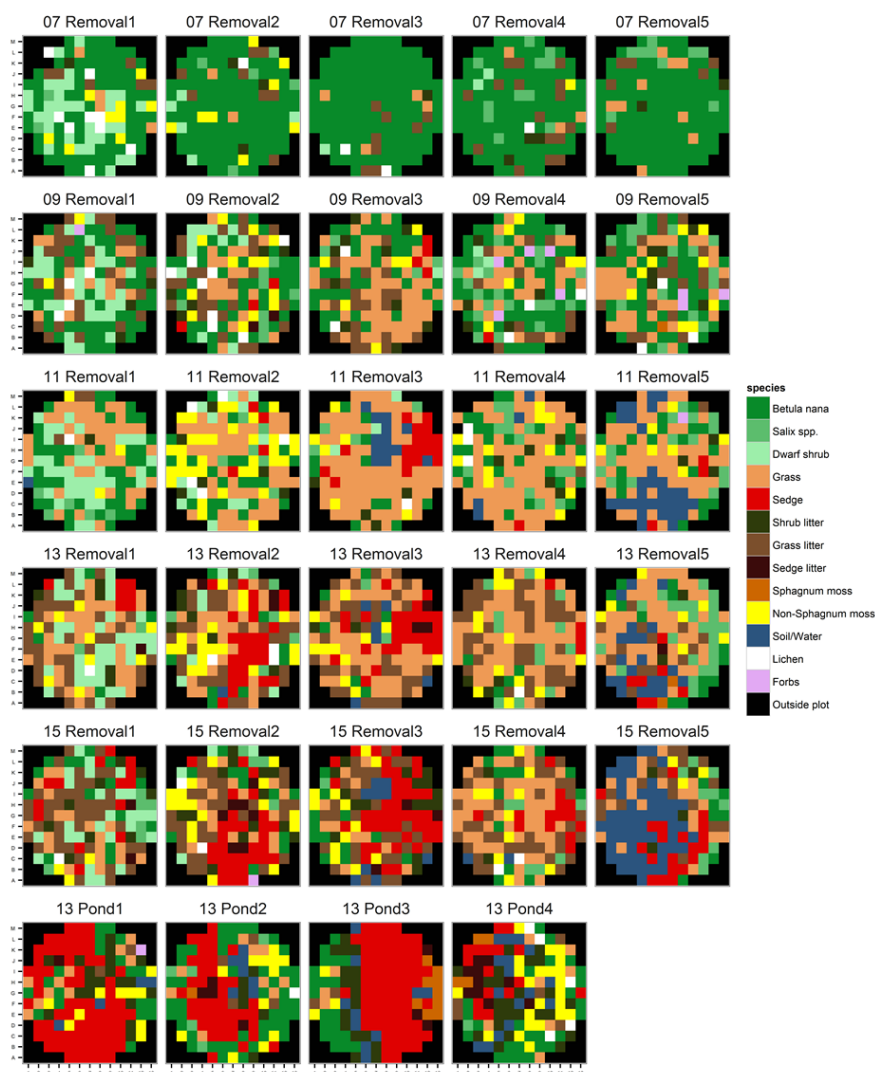
Fig. 2.6 *The vegetation composition, as seen from above the canopy, of the five B. nana removal plots and four natural thaw ponds outside the experiment area, based on the top hit of the point quadrat measurements (B. nana removal plots in 2007, 2009, 2011, 2013 and 2015 and natural thaw ponds in 2013). The colors of the mosaic represent different plant groups. The black pixels indicate the area outside the 10-m diameter plot.*

2.4.3 Comparison of removal plots with natural thaw ponds

The vegetation composition of the control plots did not change much over the years of study. In contrast, the removal plots had experienced a large vegetation shift, from *B. nana* dominated plots in 2007 before the removal treatment to plots characterized by grasses and *Salix* shrubs in 2012

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(Fig. 2.7). The cover of sedges and mud/water increased as well, indicating that the removal plots moved in the direction of the natural thaw ponds, which are characterized by the sedges, *Sphagnum* and exposed mud and water surfaces. Thaw ponds 1 and 4 seemed more similar to the removal plots, compared to the other two thaw ponds. The dendrogram providing the relationships of the vegetation compositions of the individual control, removal and natural thaw pond plots in 2013, shows that the control plots were clearly separated from the removal plots and thaw ponds (Fig. 2.A2). Although the removal plots and natural thaw ponds were still in separate groups in 2013, the removal plots had become more similar to the natural thaw ponds than to the control plots (Fig. 2.A2).
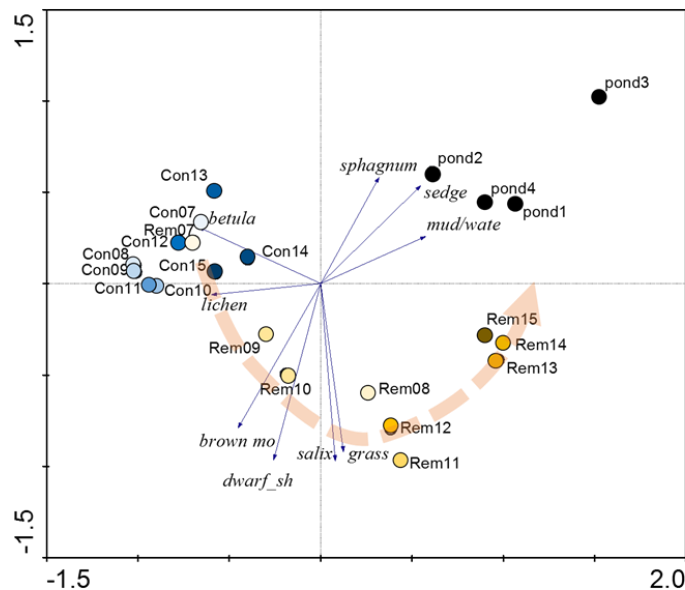
Fig. 2.7 The vegetation changes in the B. nana shrub removal experiment over time (2007-2015), based on a Principal Component Analysis of the point-quadrat data, in the control plots (blue dots) and the B. nana removal plots (orange dots). The black dots represent the four natural thaw ponds, measured in 2013. Darker color denotes the vegetation composition of the more recent years. The arrows represent the major plant groups: betula: Betula nana; salix: Salix spp.; mud/wate: exposed mud/water; dwarf_sh: other dwarf shrub (Vaccinium spp., Rubus chamaemorus and Ledum palustre ssp. decumbens); grass: grass (mainly Arctagrostis latifolia); sedge: sedge (mainly E. angustifolium); brown_mo: moss (excluding Sphagnum spp.); lichen: lichen; Sphagnum: Sphagnum spp. moss. The dashed red arrow indicates the change in the vegetation composition in the removal plots over the years.

2.5 Discussion

Although the differences in thawing depth between the control and removal plots stabilized after 2012, the removal plots continued to change. As thawing depth is measured relative to the ground

surface, the further soil subsidence after 2012 implied that the permafrost thaw in the removal plots had continued, although at a much slower pace. The increased area of standing water and exposed mud confirmed further subsidence and increasing wetness in the removal plots. Nauta et al. (2015) argued that a positive feedback loop between thawing and the increasing water area developed in the removal plots. Water is trapped in the depressions, and the pooling of water accelerated local thawing, due to the strong reduction in albedo when green or brown vegetation is replaced by the dark water surface. The collapsed concave plots also trap more insulating snow in winter, accelerating the positive feedback between permafrost thaw and soil subsidence (Nauta et al. 2015).

The vegetation composition of the removal plots shifted between 2007 and 2015 in response to the removal of *B. nana* and increasing wetness, while the vegetation composition in the control plots was rather stable. Grasses replaced *B. nana* shrubs and became the most common species in 2011 but decreased afterwards. The removal of the aboveground biomass of the highly dominant *B. nana* probably increased light and nutrient availability for the other species that were already present.

In general, soil nutrient limitation, especially of phosphorus and nitrogen, markedly suppresses the growth and the reproduction of plants in the Arctic tundra (Dormann and Woodin 2002; Shaver et al. 1998). Several nutrient addition experiments (Robinson et al. 1995; Shaver and Chapin 1986) in the Arctic tundra have shown that compared with other plant types, the grasses could benefit most from extra soil nutrients (nitrogen and phosphorus). The biomass of the grasses, which was low in the unfertilized plots, increased more than 15 fold in fertilized plots of these experiments. The fast increase of grasses, in our experiment mainly *A. latifolia,* can probably be attributed to their good ability to compete for nutrients (Kemper and Macdonald 2009).

In comparison to dwarf shrubs like *B. nana* and *V. vitis-idaea*, grasses and sedges have deeper roots (Miller et al. 1982; Shaver and Cutler 1979; Wang et al. 2016), and thus have better opportunities to reach the nutrients in the newly thawed deep soil (Oulehle et al. 2016). Low shrub canopies in tundra reduce local permafrost thawing during the growing season, maintaining shallower thawing depths than the areas dominated by other plant types (Blok et al. 2010; Wang et al. 2016). The deeper thawing depths in the removal plots imply that nutrient availability for roots growing in the deep soil has probably been higher in the removal plots. In our study, the cover of the grasses in the removal plots, which was initially below 20%, reached over 50% in the 5th year after the start of the experiment. Thus, the grass *A. latifolia* proved to be a fast responder to rapidly changing soil conditions in the removal plots, which is similar to its response in a deep soil heating and fertilization experiment at the Kytalyk site (Wang et al 2017). This grass species might be an indicator of recent abrupt thawing of the permafrost. *A. latifolia* has earlier been identified as a species that responds to disturbances (Kemper and Macdonald 2009).

Subsequently, the rapid rise of the soil moisture content and the increased area of standing water in the removal plots probably enabled the establishment and growth of sedges, mainly *E. angustifolium*, led to the decrease of the grasses, and prevented the re-growth of the *B. nana* shrubs in the removal plots simultaneously. The exceptionally high water/mud cover in 2011 in the removal plots probably resulted from the high summer rainfall (158 mm in June-August), almost

twice of the mean summer rainfall (84 mm) between 2008 and 2015. In tundra, *E. angustifolium* sedges and *B. nana* shrubs occupy areas with distinctively different soil moisture conditions. *E. angustifolium* prefers the waterlogged areas (Shaver and Chapin 1991), while *B. nana* favors the elevated, well-drained hummocks (Groot et al. 1997). The extremely high soil moisture might therefore have killed the surviving belowground stems of *B. nana* shrubs in the removal plots, stopping the regrowth of *B. nana* consequently. *E. angustifolium* was not present in the plots at the start of the experiment, but was abundant in nearby depressions surrounding the slightly elevated *B. nana* shrub patches, and perhaps needed some time to get established in the wet parts of the removal plots. It is not clear why *A. latifolia* is on the decline in the removal plots. Probably, it cannot tolerate the permanently water-saturated conditions in the thaw ponds and is therefore being replaced by *E. angustifolium*. This species is known to withstand anaerobic conditions due to its aerenchymous tissues which conduct oxygen from the atmosphere to the anaerobic root zone. Ponding water has been consistently observed in the removal plots since 2011, so we expect the sedge *E. angustifolium* to become more common in the removal plots.

The direction of the vegetation shifts was similar in all five removal plots, although the pace of the shifts differed. We suggest that the differences in present vegetation composition among the removal plots are mainly the consequence of the different substrate conditions. The ice content of the permafrost underlain lowland tundra often reaches as high as 80% by volume in the top of the permafrost (Ping et al. 2013). In our study site, the ice content in permafrost was on average 75% by volume as well (Iwahana et al. 2014). Nevertheless, the ice content in the ice-rich permafrost is not evenly distributed. The permafrost with higher ice content collapses more rapidly, as the melting of the ice drives the soil subsidence. Accordingly, the removal plots with higher ice contents in permafrost may have subsided more, resulting in higher soil moisture contents. As the tundra plants are usually sensitive to the changes in soil moisture, different soil moisture levels largely determined the variation in plant species composition of the different removal plots.

Although the removal plots are still different in comparison to the natural thaw ponds, it is clear that they resemble more and more thaw ponds 1 and 4, due to increasing sedge cover and an increased area of mud and water in the removal plots. However, *Sphagnum* cover in the removal plots is still very low compared to the thaw ponds. Perhaps these mosses represent a later succession stage, or need more time to establish, as they are not very abundant in the direct surroundings of the removal plots. We do not know how old the studied natural thaw ponds are. However, they were present in 2010 (visible on GeoEye very high-resolution satellite image). It is well possible that they have undergone the same vegetation succession trajectory of fast grass expansion at the start of thawing and soil subsidence, followed by ponding of water, *B. nana* mortality from drowning, and sedge establishment. In the natural thaw ponds, the grass is still present in-between the open water with drowned shrubs and the living *B. nana* vegetation (Fig. 2. 6). The natural thaw ponds were also characterized by the presence of *Sphagnum* moss. *Sphagnum* is known as an ecosystem engineer creating environmental conditions which halt the expansion of sedges (Heijmans et al. 2002). The moist *Sphagnum* moss carpet may provide conditions for germination and re-establishment of *B. nana* shrubs (personal observation). Ultimately, growth of ice in the aggrading permafrost under the recovering protective moss and dwarf shrub vegetation may lift the ground surface (Mackay 1995 in Streletskiy et al. 2016), creating the slightly drained

conditions needed for *B. nana* dominance.

The two thaw ponds resembling the removal plot the most are isolated thaw ponds within a shrub patch, whereas the other two thaw ponds are located along the margin of a shrub patch where the permafrost is in contact with flowing water in the adjacent drainage depression. For the isolated thaw ponds it is unknown what caused the abrupt permafrost thaw. The experiment has shown that disturbance of the vegetation can trigger increased thawing initiating a positive feedback loop of soil subsidence, trapping of snow and reduced albedo due to pooling leading to further thawing and thaw pond development (Nauta et al. 2015). Climate warming, flooding and human activities have also been suggested as possible triggers for abrupt thaw (Nauta et al. 2015; Schuur et al. 2015). In the control plots of the removal experiment the thawing depth was deepest in 2011 which was a very wet and also warm summer (Nauta et al. 2015). 2011 was also the year in which the ponding of water in the removal experiment started (Fig. 2.5), although the removal of *B. nana* regrowth in 2010 may have contributed to further thawing and soil subsidence as well. Predicted future warmer and wetter conditions could more often trigger abrupt thawing at locations where the top of the permafrost contains pure ice lenses which are not sufficiently protected by the vegetation cover.

Thaw ponds emit both CO_2 and the strong greenhouse gas methane, whereas shrub vegetation acts as a sink for both greenhouse gases (Nauta et al. 2015; van Huissteden et al. 2005). However, vegetation succession in thaw ponds will probably result in renewal of the CO_2 sink when wet sedge vegetation has developed, which may compensate for the high methane emission (Huissteden and Dolman 2012). This study has contributed new knowledge about the trajectory of thaw pond development and the initial stage of vegetation recovery, which is needed for an assessment of the greenhouse gas consequences of abrupt permafrost thaw. There are many observations of recent thermokarst, the thawing of ice-rich permafrost causing soil subsidence (many examples in Jorgenson and Grosse 2016, and Liljedahl et al. 2016). In lowlands, the soil subsidence results in new open water features such as ice wedge troughs, thermokarst pits or thaw ponds in which the pre-existing dwarf shrub vegetation drowns. It is yet unclear how widespread the phenomenon is. The coastal lowlands of eastern Siberia are known to have abundant thermokarst landforms (Olefeldt et al. 2016) and are classified as highly vulnerable to climate change (Nelson et al. 2001), yet are underreported in English-language scientific literature (Olefeldt et al. 2016). Our observations on permafrost and vegetation dynamics conducted at a Northeastern Siberian tundra site with a relatively warm summer climate could be relevant to assess future landscape changes in currently colder tundra areas as well.

2.6 Conclusion

The nine years of field observations in a unique *B. nana* shrub removal experiment at a Siberian tundra site illustrated on-going permafrost thaw, albeit at a much slower pace in recent years, and significant vegetation change after the removal of the aboveground *B. nana* biomass. The grass species *A. latifolia* appeared a fast responder to the abruptly changed light, nutrient and thawing depth conditions caused by the removal treatment. Permanent ponding of water in the subsiding removal plots, from the fifth year of the experiment, resulted in establishment of the sedge species *E. angustifolium*, which is gradually replacing the grass. The vegetation in the removal plots in the
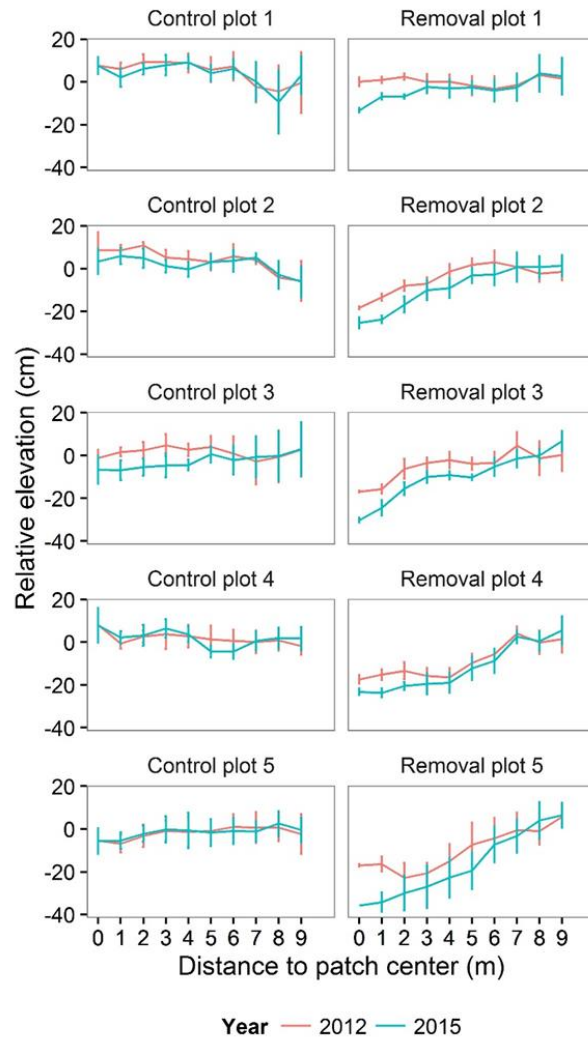
seventh year was still different compared to the natural thaw ponds at the same study site, particularly in terms of sedge and *Sphagnum* cover. However, it was clear that with the increasing sedge and open water cover the removal plots were becoming more and more similar to the natural thaw ponds in the recent years. This implies that our observations in the experiment may represent the trajectory of thaw pond development and the initial stage of vegetation recovery in lowland tundra, which is essential knowledge for an assessment of future landscape changes and the CO_2 and methane emission consequences of abrupt permafrost thaw.
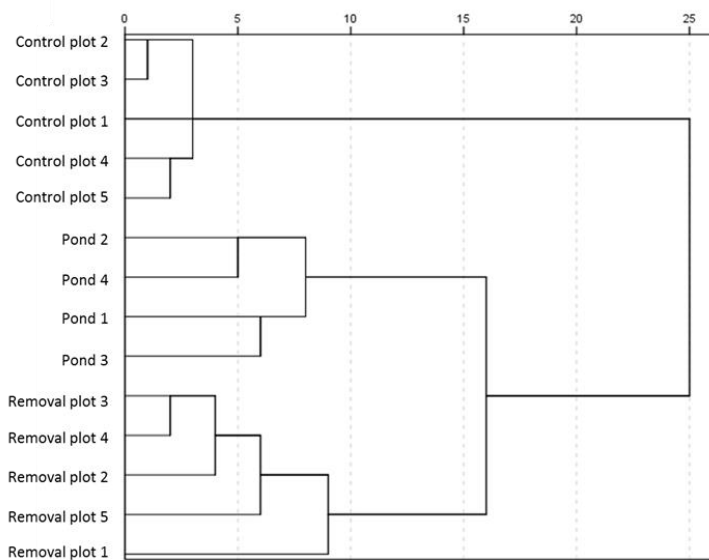
2.7 Acknowledgements

Fig. 2.A1 *Relative surface elevation of each plot in the B. nana shrub removal experiment at increasing distance from the plot centre in 2012 and 2015. Data are mean value ± s.e.m., n = 4 spots. The red lines are the relative elevation values in 2012 and the blue lines are the relative elevation values in 2015.*

Fig. 2.A2 *The dendrogram of the correspondence analysis based on the canopy vegetation composition of the plots in the B. nana shrub removal experiment (5 control plots and 5 removal plots) and the four natural thaw ponds, all measured in 2013. The closer the plots are to each other in the graph, the higher the similarity in species composition.*

Chapter 3: Peat moss carpet reduces methane emission and facilitates shrub expansion in Siberian Arctic tundra

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3.1 Abstract

Sphagnum mosses are well known as environment engineers and are widely distributed in moist northern ecosystems, i.e. boreal peatlands and Arctic tundra. They have the capability to change local vegetation distributions by creating cool and nutrient limited soil conditions. It has also been suggested that they are able to limit local methane emission as well. So far most studies focused on the ecological functions of *Sphagnum* mosses in boreal peatlands, while in Arctic tundra similar studies were rare. In this study we set up a 4-year *Sphagnum* carpet removal/addition field experiment at a north-eastern Siberian tundra site to investigate how *Sphagnum* mosses affect local abiotic conditions, vegetation dynamics and greenhouse gas fluxes. The results indicated that only a fully established *Sphagnum* cover has the capability to reduce permafrost thawing in summer. The *Sphagnum* carpet did not only limit *Eriophorum* sedge growth, but also provided a substrate for seed germination of deciduous shrub species *Betula nana* L. and *Salix* spp. The presence of *Sphagnum* mosses strongly reduced local CH_4 emission rates, probably by creating a thicker aerobic moss layer in which methane is oxidized. Local CO_2 absorption, in contrast, was strongly related to sedge tiller density. Consequently, we confirmed that the presence of *Sphagnum* mosses not only affects local abiotic environment and vegetation succession of Siberian moist tundra, but they are also pivotal to local greenhouse gas fluxes.

Key-words: *Betula nana* L., CO_2 flux, *Eriophorum angustifolium* Honck, methane flux, *Sphagnum* moss, seed germination, Siberian tundra, soil nutrient, vegetation dynamics

3.2 Introduction

The Arctic is warming at unprecedented rates, strongly influencing ecosystem structure and functioning (Hinzman et al. 2013). To date, warming has triggered large-scale permafrost degradation (ACIA 2005; IPCC 2013) and has initiated vegetation changes Arctic (Hill and Henry 2011), most notably shrub expansion (Frost and Epstein 2014; Hudson and Henry 2009; Myers-Smith et al. 2011; Shaver et al. 2001), that have the potential to further amplify atmospheric warming (Chapin et al. 2005; Eugster et al. 2000). However, at our research site in northeast Siberian lowland tundra it is unclear whether shrub expansion is taking place. We also observed local die-back of low shrub vegetation due to small-scale collapse of ice-rich permafrost leading to the formation of thaw ponds (Nauta et al. 2015). Sedge vegetation may quickly colonise these thaw ponds, followed by establishment of *Sphagnum* mosses, which may ultimately lead to the reestablishment of low shrub vegetation. Consequently, to gain insight in how Arctic ecosystems and their functions will respond to further warming, it is necessary to better comprehend the speed and direction of vegetation succession in Arctic, especially the roles of key environment engineers, such as *Sphagnum* mosses.

Sphagnum mosses are generally recognized as environmental engineers in northern wet ecosystems (Svensson 1995). *Sphagnum* species have the capacity to change their environment to a large degree, by creating a cold, acidic and nutrient poor environment that impedes the growth of vascular plant species (van Breemen 1995), such as sedges (Heijmans et al. 2002a). Conversely, the open and moist *Sphagnum* carpets also offer prime germination sites for many vascular plant species, even though most of the vascular plant seedlings are quickly overgrown by the mosses

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(van Breemen, 1995; Limpens et al., 2014). In northern peatlands, where *Sphagnum* mosses are highly dominant, the competition between *Sphagnum* mosses and vascular plants can be fierce (Pouliot et al. 2011), as *Sphagnum* mosses have been shown to strongly reduce the nutrient availability for vascular plants (Heijmans et al. 2002b). In sub-Arctic region, permafrost peatlands, *Sphagnum* mosses may off-set warming effects on shrub expansion (Lang et al. 2009), thereby maintaining moss-dominance. If *Sphagnum* could play similar roles in moist Arctic tundra where peat layers are thinner, is unknown.

Apart from influencing succession trajectories, *Sphagnum* carpets may also modify the composition and release of greenhouse gases, by its effects on production, passive release and oxidation potential of methane. The methane dampening potential of *Sphagnum* carpets can be substantial, as shown by Parmentier et al. (2011) for moist Siberian tundra. The potential of *Sphagnum* to reduce methane emissions and to facilitate establishment of low shrubs which protect the permafrost could prove essential to reduce warming effects in moist tundra. Permafrost degradation in moist tundra may increase methane emissions substantially by formation of thaw ponds (Nauta et al. 2015). Subsequent colonization of these ponds by *Sphagnum* carpets might strongly restrict local methane emissions (Treat et al. 2007).

The goal of this study was to examine the effect of *Sphagnum* carpets on soil conditions, vegetation development and greenhouse gas fluxes. In particular, we set out to test three hypotheses: 1) *Sphagnum* mosses change the local environment, i.e. decrease thawing depth, soil temperature and soil nutrient availability; 2) *Sphagnum* mosses depress the growth of sedges, while deciduous shrub seedlings can germinate in *Sphagnum* carpets; 3) *Sphagnum* mosses decrease local methane emission. To investigate these hypotheses, a three-year *Sphagnum* moss carpet removal and addition experiment was run from 2011 till 2014 in a moist lowland tundra site in northeast Siberia.

3.3 Material and methods

3.3.1 Study site

The field experiment was conducted in the northeast Siberian tundra in the Kytalyk Nature Reserve (70˚49'N, 147˚28'E), located in the Indigirka lowlands and approximately 30 km northwest of the town of Chokurdakh, Sakha Republic, Russian Federation. The whole region is underlain by over 300 m thick continuous permafrost, with a shallow active layer (less than 1 m) on top of it which thaws in summer and refreezes in winter. The mean annual temperature (1981-2010) at Chokurdakh (WMO station no. 21946 in Chokurdakh) is -13.4 °C. The mean July temperature of the same period is 10.3 °C and the mean January temperature is -34.0 °C (http://climexp.knmi.nl/). Annual precipitation is 200-250 mm, and half of it occurs during the summer months (Nauta et al. 2015).

The *Sphagnum* addition experiment was established in a drained thaw lake basin. In this former lake bed, low *B. nana* shrubs form slightly elevated shrub patches and *Eriophorum* sedges (mainly *Eriophorum angustifolium* Honck) occupy the drainage depressions and thaw ponds. *Sphagnum* moss carpets mostly occupy the transition zones between these vegetation types. Common *Sphagnum* species in the study area include *Sphagnum balticum* (Russow) C.E.O. Jensen, *Sphagnum compactum* Lam. and DC., *Sphagnum subsecundum* Nees and *Sphagnum squarrosum*

Crome. Vascular plant species such as *Salix* spp. shrubs, *Saxifraga cernua* L. and *Comarum palustre* L. are often present in the *Sphagnum* carpets.

3.3.2 Sphagnum addition experiment

Five transition zones between *B. nana* dominated shrub patches and sedge dominated depressions (Fig. 3.1) were selected for the experiment. At each location, four 1 m × 1 m plots were established, two plots (Undisturbed *Sphagnum* plots and *Sphagnum* Removal plots) in the area initially dominated by *Sphagnum* mosses, and two (Undisturbed Sedge plots and *Sphagnum* Addition plots) in the sedge dominated zones. The distances between any two plots at the same location were around 5 m. The five locations were at least 100 meters apart from each other.
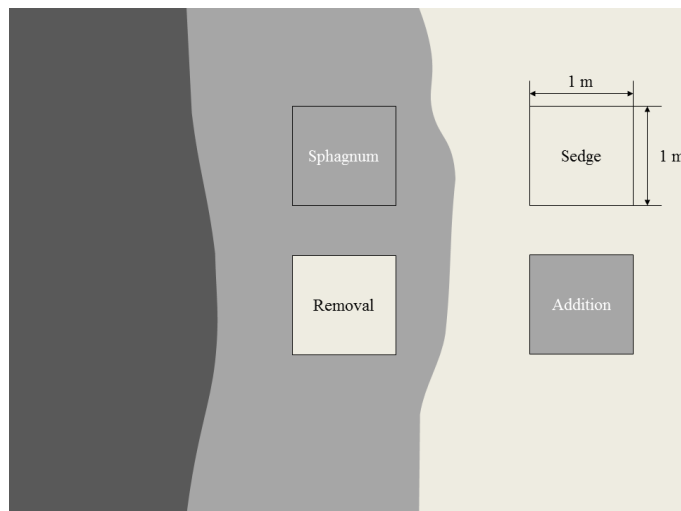
Fig. 3.1 *Graphic representation of the experiment setup: all experiment plots were located at the transition from Betula nana dominated shrub patches (dark grey) to sedge dominated depressions with standing water (light grey). The medium grey band represents the Sphagnum moss carpet with low density of vascular plants. The Sphagnum Removal plot (Removal) and the undisturbed Sphagnum plot (Sphagnum) are in the Sphagnum carpet area. The two squares in the sedge dominated area are the undisturbed Sedge plot (Sedge) and Sphagnum Addition plot (Addition). The size of each plot is 1 m × 1 m.*

In July 2011 the *Sphagnum* Removal plots were created by removing the approximately 10 cm thick mat of green *Sphagnum* mosses, leaving the brown (moss) litter layer behind. The aboveground biomass of both sedges and other vascular plants inside the *Sphagnum* Removal plots was clipped off at the brown-coloured moss litter surface after the removal of *Sphagnum* mosses. The removed *Sphagnum* moss carpets were immediately added to the neighboring *Sphagnum* Addition plots in the sedge dominated area. Only the *Sphagnum* mosses were transplanted to the *Sphagnum* Addition plots. The sedges in the *Sphagnum* Addition plots were gently bent before adding the *Sphagnum* moss layer, mimicking the invasion of *Sphagnum* mosses in Sedge dominated areas (personal observations).

3.3.3 Measurements

The active layer thickness (ALT) and water table position (WTP, the position of water surface relative to soil or moss surface) in each plot were measured approximately once a week during the growing seasons in 2012, 2013 and 2014. Soil temperature was measured with A-button temperature data loggers (Type SL52T, Signatrol Ltd, UK) buried at 15 cm below moss/soil surface. These loggers have kept running from July 2011 to July 2014. Unfortunately, due to highly wet soil conditions, more than half of the data loggers were broken during the first two years. The broken ones were replaced by new loggers with better waterproof silicon protection shells in 2013. Consequently, only the temperature data from July 2013 to June 2014 were complete enough for analysis.

Nutrient availability was assessed by burying ion-exchange resin bags. Approximately 5 g of ion exchange resins (IONAC® NM-60 H+/OH Form, type 1 beads; J.T. Baker, Philipsburg, NJ, USA) were enclosed in a 100-μm mesh size mesh bag (5 cm × 5 cm). One bag was buried at 15 cm depth in each plot in the summer 2011 and retrieved after one year (Hobbie and Chapin 1998). All resin bags were kept in cool conditions and transported to the Netherlands for nutrient analyses. The resin bags were extracted in 50 ml 2 M NaCl in 0.1 M HCl. We then used an auto-analyzer (Skalar) to measure NH_4^+, NO_3^-, PO_4^{3-} and K concentrations of the ion-exchange resins afterwards (Breeuwer et al. 2010). As the concentration of NO_3^-, is extremely low, NO_3^- was therefore not included in the further analyses.

In late July of 2013 and 2014, the sedge tiller numbers inside the 30 cm diameter PVC collar (inserted for gas flux measurement) of each plot (mainly *E. angustifolium* mixed with some *Carex aquatilis* Wahlenb) were counted for estimating the sedge density of each plot. Each plot had one collar inserted at the plot center. In late July 2014, the aboveground biomass of the sedges inside the collar area of each plot was clipped off at the moss/soil surface, dried (70 ˚C, 48 hrs in oven) and weighed.

The establishment of vascular plants in the *Sphagnum* moss carpets was assessed by counting the number of seedlings in all experimental plots in August of 2014. All the seedlings (whole plant) were collected, dried in the oven (70 ˚C, 48 hrs) and the total dry biomass of the seedlings of each plot was weighed.

The vertical increment of the *Sphagnum* moss carpet was monitored using two brush wires, a modification of the cranked wire method by Clymo (1970), erectly inserted into each plot at the start of the experiment in 2011 (Breeuwer et al. 2010; Limpens et al. 2004). We measured the relative moss/soil surface twice (at July, 2011 and early August 2014, the beginning and the end of the experiment).

Greenhouse gas fluxes (CO_2, CH_4) were measured four times (8th, 18th, 26th and 29th of July, 2013) by using chamber measurements (Ultraportable Greenhouse Gas Analyzer, Los Gatos research, USA). The size of the transparent cylinder chamber was 28 cm high and 30 cm in diameter. For the chamber measurements, one 15 cm high PVC collar with the same diameter of 30 cm was half buried at the center of each plot at the beginning of July, 2013 and kept at the same location afterwards. The chamber was carefully put on the collar, creating an air-tight chamber space during

the measurement. Each gas flux measurement lasted approximately 2-3 minutes, and the gas analyzer recorded the chamber gas concentrations once per second (120-180 records per measurement). Based on the linear increase or decrease of CH_4 and CO_2 concentrations together with measured soil temperature at 5 cm depth and chamber height (above soil surface), greenhouse gas fluxes of each plot were calculated in Matlab (version R2013a, Mathwork Inc., USA).

To explore potential treatment artifacts, e.g. disturbance due to the removal or addition treatment, on environmental or biotic responses, we selected 11 isolated *Sphagnum* patches (from 1 m^2 to approximately 20 m^2) newly formed within sedge dominated drainage depressions in the beginning of August, 2014. Within each patch, we randomly selected a 50 cm × 50 cm large plot and one same size plot outside the patch in the neighboring sedge dominated area for comparison. The numbers of both sedge tillers and deciduous shrub ramets in each plot were recorded. In addition, the ALT and WTP of each plot were measured at the same time using the same methods as in the experiment.

3.3.4 Statistical Analyses

The statistical analyses were performed in SPSS software (IBM SPSS Statistics for Windows, ver. 19.0; IBM Corp., Armonk, NY, USA). During the tests, the residual normality of every variable was tested. The gas flux data were ln-transformed to improve the homogeneity of variances and residual normality. For the measurements that were done in multiple years (ALT, WTP, sedge tiller density) or on multiple dates (gas fluxes), we used repeated measures analysis of variance (RM-ANOVA) combined with Tukey's-b post-hoc test to detect significant differences between the plots of the four treatments (Undisturbed Sedge plots, *Sphagnum* Addition plots, Undisturbed *Sphagnum* plots and *Sphagnum* Removal plots).

We further used one-way ANOVA combined with Tukey's-b post-hoc test to rank the key soil nutrient availability (ammonia-nitrogen and phosphate-phosphorous) differences among the four treatments. Location, the five transition zones, was not included as a random factor in the ANOVAs, as it did not change the general results. Spearman's correlation tests were applied to explore relationships between sedge tiller density and environmental variables (ALT, WTP, NH_4^+ and PO_4^{3-} availability); and relationships between gas fluxes and sedge tiller density and environmental variables.

At last, the ALT, WTP, sedge tiller density and non-sedge vascular plants numbers in plots both inside and outside the 11 isolated *Sphagnum* patches were compared by using paired t-tests.

3.4 Results

3.4.1 Abiotic factors

The ALT increased progressively across the growing seasons of 2012 (time: F = 170.4, p <0.01) and 2013 (time: F = 47.7, p < 0.01). In both years of analysis (2012, 2013) ALT was lowest in the Undisturbed *Sphagnum* plots (Fig. 3.2a, b). Removal of the *Sphagnum* mosses strongly increased the ALT (24.0 ± 1.3 cm and 31.8 ± 1.0 cm in Undisturbed *Sphagnum* and *Sphagnum* Removal plots respectively in 2012; 22.9 ± 2.0 cm and 35.2 ± 1.5 cm in 2013). However, also addition of *Sphagnum* mosses increased the ALT (30. 7 ± 1.4 cm and 35.8 ± 4.0 cm in Undisturbed Sedge and *Sphagnum*

Addition plots respectively in 2012; 29.0 ± 1.2 cm and 34.7 ± 1.4 cm in 2013). This is in agreement with the measurements outside the experiment in which the ALT inside the isolated *Sphagnum* patches within the sedge zone was significantly larger than the ALT outside these patches (39.5 ± 1.5 cm and 48.3 ± 2.5 cm outside and inside the isolated *Sphagnum* patches), suggesting that the *Sphagnum* Addition treatment effect on ALT could not be solely attributed to manipulation artifacts.

WTP was the highest in the Undisturbed Sedge plots, both in 2012 and 2013, with water level above the soil surface in 2012. The Undisturbed *Sphagnum* plots had the deepest WTP in 2013 (Fig. 3.2c, d). In the isolated *Sphagnum* patches, similarly, WTP values were much lower inside these *Sphagnum* patches (-14.5 ± 1.2 cm) than outside the *Sphagnum* patches (7.9 ± 1.1 cm).
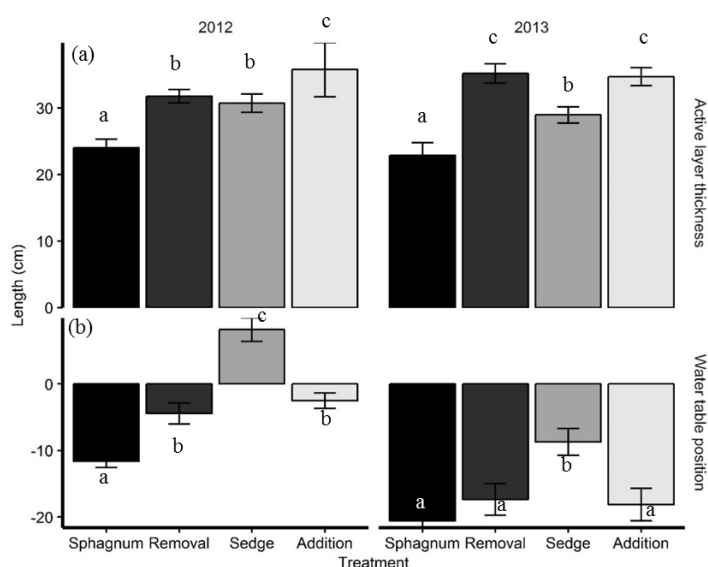
***Fig. 3.2** ALT (active layer thickness, cm) during the growing season of 2012 (a) and 2013 (b); WTP (water table position relative to moss/soil surface, cm) during the growing season of 2012 (c) and 2013 (d). Different letters (a, b, c) represent significant differences among the within-patch positions.*

The measured soil nutrient exchangeable concentrations (ammonia-nitrogen and phosphate-phosphorus) were not significantly different among the four treatments. This was probably related to large variability among the plots within the treatments. However, the mean exchangeable nutrient concentrations tended to be higher in the plots without a *Sphagnum* carpet (*Sphagnum* Removal and Undisturbed Sedge treatment).

The mean soil temperature at 15 cm depth measured from July 13 till August 31, 2013 showed similar patterns among the four treatments. *Sphagnum* Removal and *Sphagnum* Addition plots had the highest soil temperature over this period, while the other two plot types had lower mean temperature across the period, but the differences between the treatments became smaller over the measurement period (time: $F = 238.6$, $p < 0.01$; time × treatment: $F = 13.9$, $p < 0.01$, Fig. 3.A2).

3.4.2 Biotic factors

Sedge tiller number was strongly reduced by the *Sphagnum* Addition treatment (number = 38 ± 4 in 2013 and number = 48 ± 15 in 2014) compared to their control plots (Undisturbed Sedge plots) (number = 212 ± 31 in 2013 and number = 274 ± 36 in 2014), both in 2013 and 2014 (Fig. 3.3a). The same effect of *Sphagnum* Addition was apparent for sedge aboveground biomass, which was measured only in 2014 (Fig. 3.3b). *Sphagnum* removal did not change sedge tiller number compared to the Undisturbed *Sphagnum* plots (Fig. 3.3a). However, sedge aboveground biomass was significantly increased by the *Sphagnum* removal treatment (Fig. 3.3b). Neither sedge tiller number, nor sedge aboveground biomass were correlated with soil exchangeable ammonia-nitrogen (number in 2013: $r = 0.27$, $p = 0.27$, $n = 20$; number in 2014: $r = 0.23$, $p = 0.34$, $n = 20$; biomass: $r = 0.06$, $p = 0.80$, $n = 20$, Fig. 3.3c) or phosphate concentrations (number in 2013: $r = 0.32$, $p = 0.17$, $n = 20$; number in 2014: $r = 0.23$, $p = 0.33$, $n = 20$; biomass: $r = 0.25$, $p = 0.29$, $n = 20$, Fig. 3.3d).

B. nana and *Salix* spp. seedlings were found in the summer of 2014. Almost all of them were observed in the *Sphagnum* dominated plots (7 *Salix* spp. and 12 *B. nana* seedlings in Undisturbed *Sphagnum* plots; 7 *Salix* spp. and 2 *B. nana* seedlings in *Sphagnum* Addition plots), while the other plots without a *Sphagnum* carpet hardly had any shrub seedlings (only 2 *Salix* spp. seedlings were found in one *Sphagnum* Removal plot, Fig. 3.A3). In addition, it was observed that deciduous shrub plants (either *B. nana* or *Salix* spp.) were found in 8 out of the 11 investigated isolated *Sphagnum* patches, while no deciduous shrub plants at all were found in the sedge-dominated plots outside these patches.
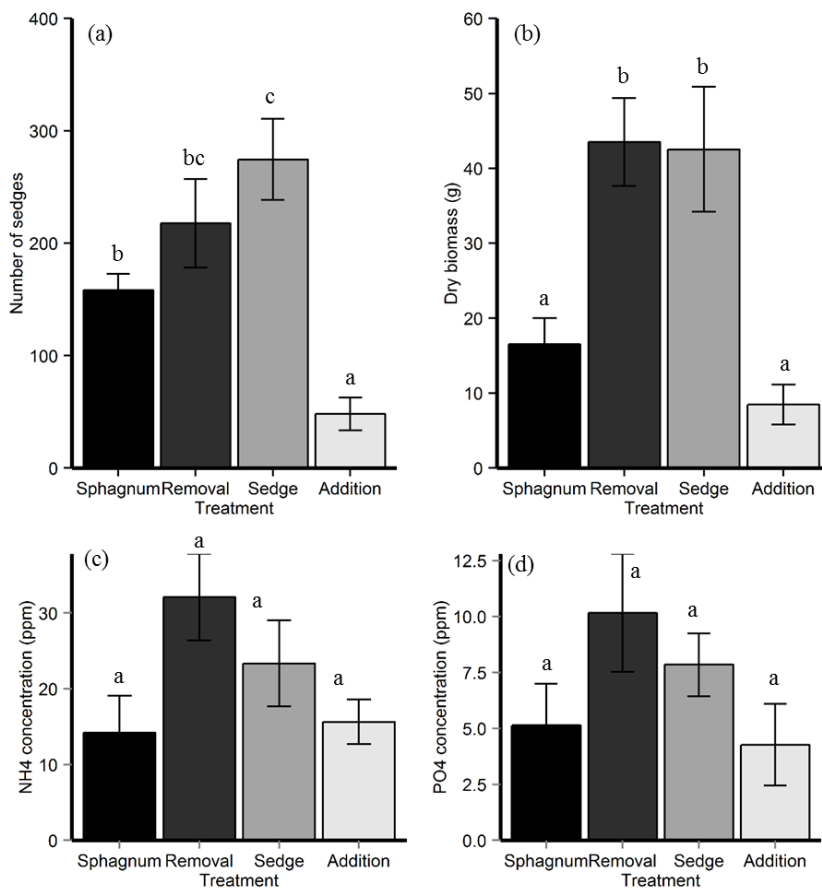
Fig. 3.3 *Tiller number (a), sedge dry biomass (b), NH_4^+ concentration (c) and PO_4^{3-} concentration (d) of the four different plot types in 2014 of a different plot types (Addition (black), Sedge (dark grey), Removal (medium grey) and Sphagnum (light grey) plots). Different letters (a, b, c) represent significant differences among the within-patch positions.*

3.4.3 CH₄ and CO₂ fluxes

The patterns of CO_2 and CH_4 fluxes were distinctively different. Measured CO_2 fluxes were always negative, reflecting a net uptake of CO_2 by the vegetation, while CH_4 fluxes were positive (Fig. 3.4), indicating a net emission of CH_4 to the atmosphere. In the Undisturbed Sedge, *Sphagnum* Removal and Undisturbed *Sphagnum* plots, CO_2 fluxes across the growing season decreased progressively, while in *Sphagnum* Addition plots no such trend could be observed (Fig. 3.4).
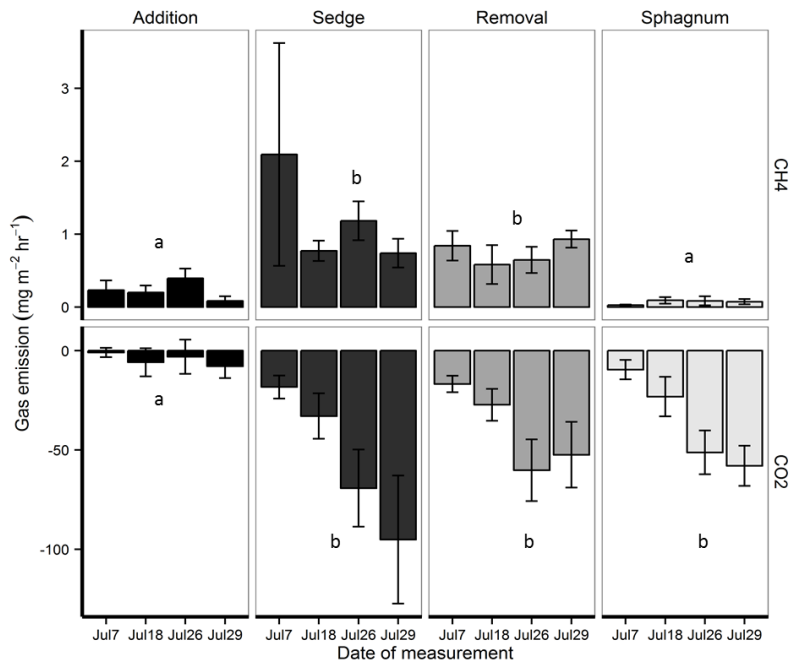
Fig. 3.4 *The mean gas fluxes(A: CH$_4$ flux; B: CO$_2$ flux) of four different plots (Addition (black), Sedge (dark grey), Removal (medium grey) and Sphagnum plots (light grey)) in July 8, 18, 26 and 29, 2014. The error bars reflect the standard errors. Negative gas fluxes indicate net absorption/uptake, whereas positive fluxes indicate net emission. The "a" and "b" letters represent different groups of gas fluxes based on the RM-ANOVA and Post-hoc tests.*

The two treatments with a *Sphagnum* carpet (*Sphagnum* Addition plots and Undisturbed *Sphagnum* plots) had much lower CH$_4$ emission rates than the two treatments without a *Sphagnum* carpet (Undisturbed Sedge plots and *Sphagnum* removal plot, Fig. 3.4). The series of correlation tests showed that CH$_4$ emission rate was correlated with plot WTP on two dates of measurement ($r = 0.72$, $p < 0.01$ at July 8; $r = 0.49$, $p = 0.04$ at July 29).

Sphagnum Addition plots had by far the lowest CO$_2$ uptake (Fig. 3.4). There hardly were any correlative relationships between CO$_2$ uptake and either WTP or ALT. However, CO$_2$ fluxes were significantly correlated with sedge tiller numbers on all dates of measurement (r between -0.67 and -0.55, $p \leqslant 0.01$).

3.5 Discussion

3.5.1 Sphagnum and its influence on abiotic factors

It proves that established *Sphagnum* carpets truly mitigate local permafrost thawing, while the newly formed *Sphagnum* carpets hardly alleviate permafrost thawing during the growing season. On the one hand, local soil temperature and active layer thickness obviously increased if

undisturbed *Sphagnum* cover was removed, which reflected the soil cooling effect of *Sphagnum* cover, as addressed by van Breemen (1995). Without the protection of *Sphagnum* carpet, soil temperature is increasing obviously. On the other hand, permafrost thawing was not alleviated in the *Sphagnum* Addition plots (Fig. 3.2), indicating that newly formed *Sphagnum* cover did not show cooling capacity. In the naturally formed 11 isolated *Sphagnum* patches we investigated, the ALT of these patches were also obviously larger than the nearby sedge dominated plots (Fig. 3.A1). It is likely that the ice accumulation under *Sphagnum* mosses is a time consuming process, which explains why the newly formed *Sphagnum* patches still had thick active layer during the growing season.

3.5.2 Sphagnum and greenhouse gas fluxes

We found in this study that *Sphagnum* carpets largely influenced the CH_4 gas emission, probably by creating thicker aerobic moss layers. *Sphagnum* mosses pronouncedly decreased the July CH_4 emission, both in the newly formed *Sphagnum* carpets (compared to Undisturbed Sedge plots) and in existing Undisturbed *Sphagnum* carpets (compared to *Sphagnum* Removal plots). It is likely that the plots with *Sphagnum* cover (Undisturbed *Sphagnum* plots and *Sphagnum* Addition plots) have thicker aerobic moss layers, in which methane is oxidized. Unsaturated *Sphagnum* carpets are perfect for survival of endophytic CH_4-oxidizing bacteria (Kip et al. 2010; Raghoebarsing et al. 2005). These microorganisms can live inside and in-between the cells of *Sphagnum* mosses. After CH_4 gas produced in deeper anaerobic soil is oxidized to CO_2 by the bacteria, the transformed CO_2 gas can be consumed by *Sphagnum* mosses for photosynthesis (Raghoebarsing et al. 2005).

Soil temperature and active layer thickness were not positively correlated with methane fluxes in the *Sphagnum* Addition plots. The *Sphagnum* Addition plots with new *Sphagnum* carpets had both higher summer soil temperatures and thicker active layers, compared with the control plots (Undisturbed Sedge plots), still methane emission rates in the *Sphagnum* addition treatment were only 10-30% of those in the Undisturbed sedge plots. Moreover, the measured methane fluxes were not significantly related to the sedge density (*r* between 0.11 and 0.35, *p* from 0.13 to 0.65, *n* = 20) as well. The lack of a seasonal development in CH_4 fluxes, which is apparent in the CO_2 fluxes, also indicates that the development of sedges during the growing season did not much influence the methane flux. Accordingly, the sedge density appeared not an influential factor that controls local gas emission.

Oppositely, it was clear that *Eriophorum* sedge density was pivotal to CO_2 absorption given its constantly high negative correlation with CO_2 flux across the growing season. Compared with *Sphagnum* mosses, the photosynthetic capability of *Eriophorum* sedges is much higher. Sonesson et al (1980) stated that the photosynthetic rates of the bryophytes such as *Sphagnum* mosses is merely one tenth to one fifth of the rates of vascular plants. Still net carbon uptake in *Sphagnum* carpets takes place, probably because of the low soil respiration rates related to the extremely low decomposition rates of *Sphagnum* litter. In this study, the number of sedge tillers largely determined the rate of CO_2 absorption. We also observed progressive increments of CO_2 absorption rates in all treatments except for the *Sphagnum* Addition treatment which had the lowest sedge tiller density. During the period of July 8 to July 29, the leaf biomass accumulation of sedges was probably fast, and the total amount of chlorophyll is probably at the peak at the end of

July. Therefore, the increasing CO_2 absorption rate over the growing season is likely caused by the development of sedge leaves (Peichl et al. 2015).

3.5.3 Sphagnum as suppressor of sedge growth

This study suggests that *Sphagnum* mosses limit local sedge growth, but it could not be shown that the sedge growth reduction was because of restricted local nutrient availability.

Sedge biomass was much higher in the plots without *Sphagnum* carpets than with *Sphagnum* carpets. It corresponds with the situation in the isolated *Sphagnum* patches of this area (Fig. 3.A1). Van Breemen et al. (1995) argued that *Sphagnum* mosses can largely suppress the growth of sedges by creating a nutrient poor soil condition, since *Sphagnum* not only efficiently intercepts the nutrient from water (Svensson 1995) and atmosphere (Heijmans et al. 2002b; Malmer et al. 1994), but also slows down the decomposition of both *Sphagnum* moss litter and *Eriophorum* sedge litter (Hájek et al. 2011). In *Sphagnum*-dominated bogs, poor N and P supply is often the key factor that hampers the aboveground productivity of vascular plants in peat bogs (Hayati and Proctor 1991; Heijmans et al. 2002a; Limpens et al. 2003). Nevertheless, in this study *Sphagnum* carpets did not significantly decrease local soil nutrient availabilities (Fig. 3.3c and d). Therefore, the mechanism that *Sphagnum* mosses suppress sedge growth is still unclear.

3.5.4 Sphagnum as facilitator of B. nana shrub establishment

Sphagnum mosses, furthermore, appeared a suitable substrate for deciduous shrub germination in this study, as we observed *B. nana* and *Salix* seedlings almost exclusively in plots with a *Sphagnum* surface (Fig. 3.A3). Our observations of seedlings proved that seed germination of *B. nana* existed in both fully established *Sphagnum* covers (Undisturbed *Sphagnum* plots) and newly formed *Sphagnum* carpets (*Sphagnum* Addition plots). In *Sphagnum* Addition plots, the *B. nana* seedlings were observed in the third growing season since the initiation of the experiment, referring to a fast invasive attempt of *B. nana* shrubs. Furthermore, outside the experiment, in the 11 isolated *Sphagnum* plots we investigated, more than half of them had deciduous shrubs (*B. nana* and *Salix* spp. ramets). Since these *Sphagnum* patches were far away from any deciduous shrub patches, the individuals we found inside these patches were very likely from seed germinations.

The *Sphagnum* mosses probably created the moist but unsaturated substrate that is needed for shrub seedlings and seedling establishment (Oberbauer and Miller 1982). Small seeds with little carbohydrate reserves can hardly produce deep roots to reach soil water in a drying environment with extremely short growing seasons (Billings and Mooney 1968). The mean weights of *B. nana* seeds are merely between 0.1 mg and 0.3 mg (Groot et al. 1997), which means that the seeds require proper moist conditions for germination. Unlike *B. nana* dominated patches and sedge dominated depressions, the areas in-between occupied by *Sphagnum* mosses have high soil moisture but are not fully water-saturated. Similarly, the isolated *Sphagnum* patches of our study also had an obvious unsaturated layer between water table and *Sphagnum* surface, while the adjacent sedge dominated area usually had standing water. Although studies focusing on *B. nana* germinations in *Sphagnum* cover are rare, many researches on conifer seed germinations in *Sphagnum* dominated bogs showed high germination rates of woody plants. The study of

Gunnarsson and Rydin (1998) in a Swedish bog showed that more than 75% of conifer seeds germinated in *Sphagnum* carpets, compared to < 5% germination rate in boreal forests (Zackrisson et al. 1997). It was also found that over 90% of established trees originally germinated in *Sphagnum* carpets (Ohlson et al. 2001). Accordingly, it is reasonable to suggest that *Sphagnum* carpets in moist tundra are ideal locations for seed germinations of *B. nana*.

It is still far from clear whether the establishment of full-grown *B. nana* shrubs will occur in current *Sphagnum* dominated areas in the future (Peichl et al. 2015). *Sphagnum* mosses in northern ecosystems can easily bog down vascular plant seedlings (Gunnarsson and Rydin 1998; Ohlson et al. 2001; van Breemen 1995). Thus, the mortality of seedlings sometimes reaches 100% in peat bogs (Gunnarsson and Rydin 1998; Ohlson 1999). In our experiment, the height increment of *Sphagnum* was small (7.6 ± 0.8 mm in *Sphagnum* plots and 15.0 ± 1.5 mm in Addition plots in 3 years) and many adult *B. nana* shrubs can be found on *Sphagnum* carpets in our research site. Therefore, the probability that *B. nana* seedlings will be overgrown by *Sphagnum* mosses in Arctic tundra seems relatively low.

3.6 Conclusion

As an environment engineer species, *Sphagnum* is known to play an important role in both the vegetation dynamics and local abiotic environment in boreal peatlands. At our study site in the Arctic tundra of northeast Siberia, the presence of *Sphagnum* mosses not only largely suppressed the growth of sedges, but probably facilitated the expansion of shrub dominated territories as well. Our study further suggests that *Sphagnum* mosses decrease CH_4 emission by creating thicker unsaturated moss layer and may restrict the local CO_2 absorption by limiting sedge growth. Further investigations are needed to quantify the possible shrub establishment on *Sphagnum* carpets to clarify the role of *Sphagnum* in shrub expansion in moist lowland tundra.

3.7 Acknowledgements
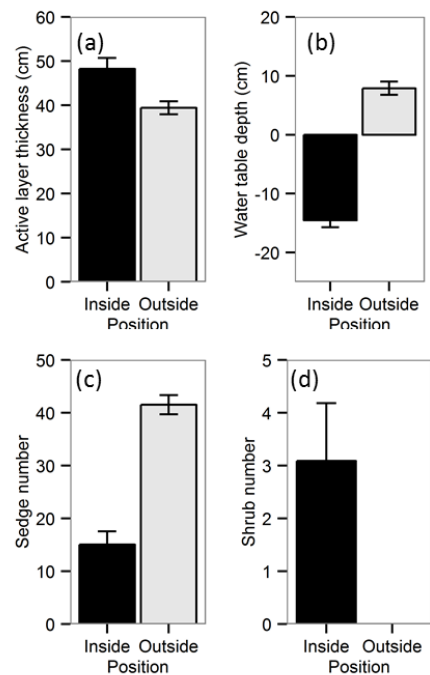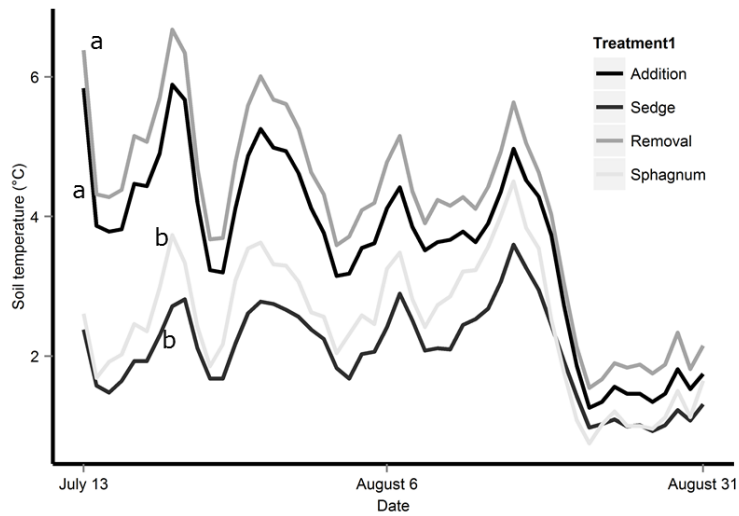
Fig. 3.A1 *The Active layer thickness (a), Water table position relative to the moss/soil surface (b) Sedge tiller number (c), and Deciduous shrub number (d) in the 50 cm × 50 cm plots inside and outside the 11 isolated Sphagnum moss patches*

Fig. 3. A2 *Daily mean soil temperature at 15 cm depth of four different plot types (Sphagnum Addition (Addition), Undisturbed Sedge (Sedge), Sphagnum Removal (Removal), Undisturbed Sphagnum (Sphagnum)) averaged over 5 replicate plots. The "a" and "b" letters represent different groups of soil temperature during this period based on the RM-ANOVA and Post-hoc tests.*
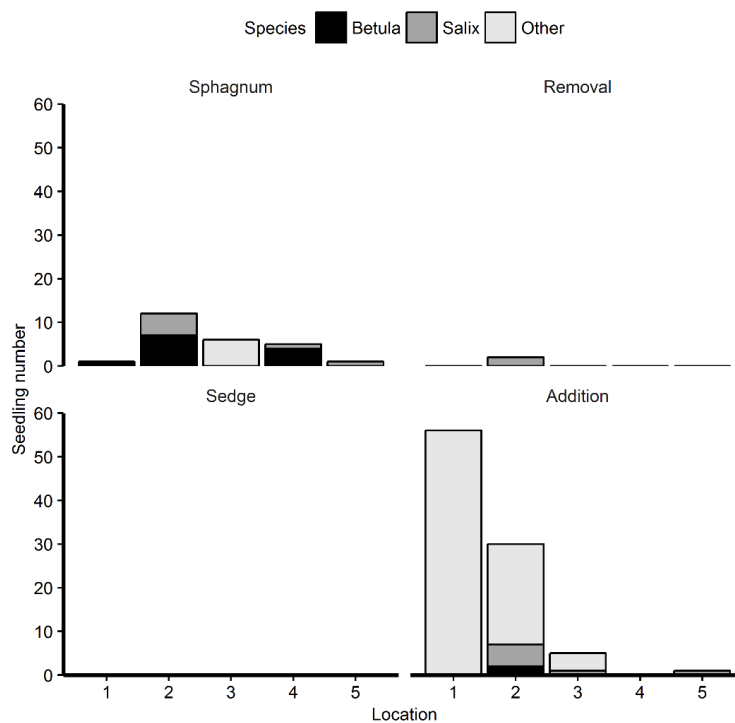
Fig. 3.A3 *Seedling numbers of vascular plant seedlings (excluding sedges) in the different plot types (Sphagnum Addition (Addition), Undisturbed Sedge (Sedge), Sphagnum Removal (Removal) and Undisturbed Sphagnum (Sphagnum)).*
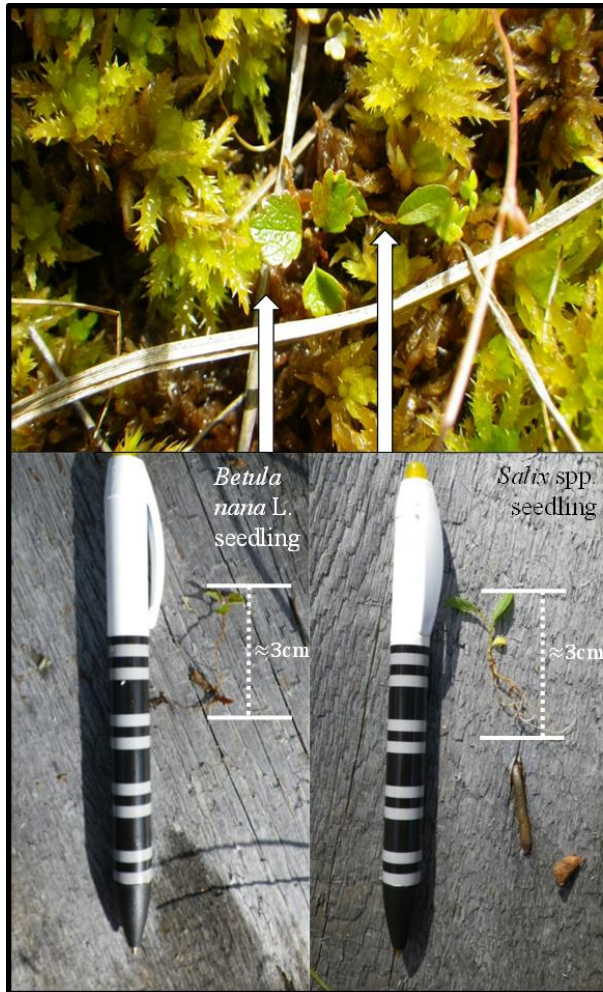
Fig. 3.A4 *The deciduous shrub seedlings Betula nana (left) and Salix spp. (right) inside one of the Sphagnum Addition plots. The photos were taken at the end of July, 2014.*

Chapter 4: Sexual reproduction and establishment of *Betula nana* at a Northeastern Siberian tundra site

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4.1 Abstract

Climate-driven shrub expansion has been widely observed and studied in the Arctic tundra, but only a few studies focused on the reproduction and establishment of the shrubs in this ecosystem. In this study, we explored how the widespread Arctic deciduous shrub species *Betula nana* reproduces and forms communities at a Siberian lowland tundra site, using molecular tools (microsatellites) and dendrochronological methods. The dendrochronology work did not support the assumption that the *B. nana* ramets vegetatively spread from center to edge within *B. nana* dominated patches, suggesting that vegetative reproduction is restricted. The results of the microsatellite analyses suggest that, instead, sexual reproduction of *B. nana* by seeds is prevalent in the study area. Moreover, gene flow of *B. nana* is not hampered by short geographic distances, implying that *B. nana* seeds can be easily transported to potential suitable substrates for germination, but low summer precipitation may limit seedling establishment. Warming of the Arctic tundra probably increases the sexual reproduction rate of *B. nana*, but may also lead to increased mortality due to more frequent local permafrost collapse. Accordingly, the question remains as to whether continuous warming will result in significant shrub expansion in lowland Arctic tundra landscapes that are vulnerable to thermokarst development.

Key words: Arctic lowland tundra, *Betula nana* L., sexual reproduction, microsatellite, dendrochronology, shrub expansion

4.2 Introduction

Rapid climatic warming in the past 50 years (IPCC, 2013) has induced vegetation changes in the Arctic tundra (Williams et al., 2000), and shrub expansion is one of the most noticeable phenomena. This observation is mainly based on repeat aerial photography (Tape et al., 2006; Hudson and Henry, 2009; Osterkamp et al., 2009) but also supported by trends observed with satellite imagery (Goetz et al., 2005; Bunn and Goetz, 2006). Such assessments are primarily from Alaska (Stow et al., 2004; Tape et al., 2006; Osterkamp et al., 2009) and Canada (Hudson et al., 2011; Myers-Smith et al., 2011), but also from northern Siberia (Frost and Epstein, 2014). Paleoecological records by Brubaker et al. (1995) showed that warmer climate along with shrub expansion has probably been a recurrent phenomenon in the Arctic tundra. Multi-year field experiments (Shaver et al., 2001; Tape et al., 2006; Bret-Harte et al., 2008; Myers-Smith et al., 2011) also suggested that especially deciduous shrub species, like dwarf birch *Betula nana* L., gained more aboveground biomass and became more dominant in the Arctic tundra under warmer conditions with higher nutrient availability.

The observed contemporary rapid shrub expansion in the Arctic tundra can be the result of 1) enlargement of existing shrub individuals, 2) lateral clonal expansion of existing shrub communities, and/or 3) enhanced sexual recruitment of new shrub seedlings (Myers-Smith et al., 2011). It is widely agreed that vegetative reproduction, i.e. lateral clonal expansion, instead of sexual recruitment is predominant in the Arctic tundra (Billings, 1987), mainly because short and unpredictable growing seasons are often detrimental to seed production and seed quality (Bliss, 1971). High levels of soil disturbance from erosion and cryoturbation can moreover make seedling establishment highly perilous (Bliss, 1971; Callaghan and Collins, 1976; Jónsdóttir et al., 1996).

While a few recent researches using genetic tools on the perennial plants of the Arctic tundra questioned this statement (Steltzer et al., 2008; Douhovnikoff et al., 2010), genetic studies on Arctic shrub reproduction are still sparse. More concrete supportive information therefore is needed to determine the rate of sexual versus clonal reproduction and driving factors behind both reproduction modes.

Potential shifts from vegetative reproduction to sexual recruitment may accelerate shrub cover expansion (Myers-Smith et al., 2011). Vegetative reproduction enables the shrub to expand via gradual belowground extension. Sexual reproduction by seeds however enables shrubs to colonize new areas beyond the existing shrub communities. In case of the wind-dispersing deciduous shrub species birch and willow, seeds can easily be transported over a long distance. Yet, the links between the reproduction mode and the shrub expansion process have been poorly studied, even though this is important to better understand and predict the vegetation shifts in the warming Arctic tundra.

Accordingly, we tried to unravel the reproduction mode of the *B. nana* shrub at a lowland tundra site in Northeastern Siberia. In a drained thaw lake basin at this site, *B. nana* is dominant on slightly elevated permafrost mounds (Siewert et al. 2015). These *B. nana* dominated patches provide a good opportunity to explore how *B. nana* dominated shrub communities developed and established. More specifically, we 1) assessed genetic variation within and between shrub communities dominated by *B. nana* using microsatellites to explore the predominant reproduction mode (i.e., vegetative reproduction or sexual recruitment) 2) determined age patterns within shrub patches using dendrochronology (age determination by counting shrub rings), to explore how these *B. nana* patches expanded. We proposed vegetative reproduction to be the predominant mode of shrub patch expansion. Consequently we expected low levels of genetic variation within shrub patches, some genetic differentiation between patches and a clear age pattern with oldest *B. nana* ramets located in the center of the patches and the youngest ramets found along the margins of each shrub patch.

4.3 Material and methods

4.3.1 Site description

The study area is situated in the Kytalyk Nature Reserve (70˚49′N, 147˚28′E), approximately 30 km northwest of the town of Chokurdakh, Yakutia, Russian Federation (Fig. 4.1). The research site is located in the lowlands of the Indigirka River in Northeastern Siberia. This region is characterized by thick continuous permafrost with a shallow active layer (maximum less than 50cm) – soil that thaws each summer. Our study area was a drained thermokarst lake bed and an adjacent Pleistocene remnant area called "ridge", which is approximately 20-30 m higher than the lake bed (van der Molen et al., 2007).

Fig. 4.1 *The mosaic-like Betula nana patches at a Northeastern Siberian tundra site (near Kytalyk research station, Sakha republic, Russian Federation, 70˚49'N, 147˚28'E). The dark green patches are dominated by B. nana shrubs, and the areas with light green or yellow are mainly occupied by Eriophorum spp. sedges and Sphagnum mosses.*

The vegetation of the research area is classified as a moist tussock sedge dwarf-shrub moss tundra (G4; the Circumpolar Arctic Vegetation Map, Walker et al., 2005). The vegetation includes deciduous shrub species such as dwarf birch *B. nana*, and dwarf willow *Salix pulchra* Cham., evergreen shrubs like *Vaccinium vitis-idaea* L*., Rhododendron subarticum* Harmaja, *Eriophorum* graminoids and *Sphagnum* mosses. In the lake bed, *B. nana* is dominant on relatively dry and slightly elevated areas. These shrub patches are surrounded by a diffuse drainage network of wet depressions dominated by the sedge species *Eriophorum angustifolium* Honck. (Fig. 4.1). On the adjacent ridge, the vegetation is more mixed: *B. nana* co-occurs with *E. vaginatum* L., *V. vitis-idaea* and *R. subarticum* (Blok et al., 2010). The ridge is approximately 20-30 m higher than the lake bed surface (Wang et al., 2016). *B. nana* shrubs cover approximately 20% of the land territory in this region (Blok et al., 2011).

The meteorological data from 1945 to 2015 was available from the closest meteorological station in the town Chokurdakh, 30 km south of the site (http://climexp.knmi.nl/). The mean annual temperature is around -13.4 $^\circ$C (1981-2010) with an average temperature of -34.0 $^\circ$C in January and of 10.3 $^\circ$C in July (Nauta et al., 2015). The growing season is defined as the snow free period and lasts from early June to the end of August.

4.3.2 Leaf sampling and microsatellite analysis

<u>Leaf sampling strategy</u>

In 2013, leaf samples for genetic analyses were collected along a 700 m long transect from the elevated ridge into the lake bed (Fig 4.2). Focal sample locations were selected at regular intervals (at least 50 m) along the transect. At each sample location a pair of *B. nana* patches was selected, with an average distance of c. 5 m between the 2 individual patches. The radius of the patches was on average 10 m. Within each patch, three transects (from center to patch edge) located at an angle of 120 degrees were established. Along each transect, 5 evenly distributed within-patch positions were selected, and 3-4 leaves from the same *B. nana* stem were collected at each within-patch position. In total 7 pairs of patches were selected along the ridge-lakebed transect (1 pair on the ridge and 6 pairs in the lake bed). On the ridge *B. nana* shrubs do not form distinct shrub patches. We therefore selected two 10 m radius area (around 50 m away from each other, patch G1 and patch G2) where we mimicked the sampling strategy used in the lake bed. In addition, 10 *B. nana* leaf samples were collected at the Kodak Station (70.56 $^{\circ}$N, 148.26 $^{\circ}$E) c. 42 km south of our focal study area (Liang et al., 2014) in the summer of 2014. Hereby, 10 samples were collected at 10 m intervals to minimize the chance of collecting vegetative ramets (patch H). Overall, this sampling design resulted in 220 *B. nana* plants in total. We treated the 15 patches/locations as 15 distinctive populations (A1, A2, B1, B2, C1, C2, D1, D2, E1, E2, F1, F2, G1 and G2 at our focal site and H in Kodak station).
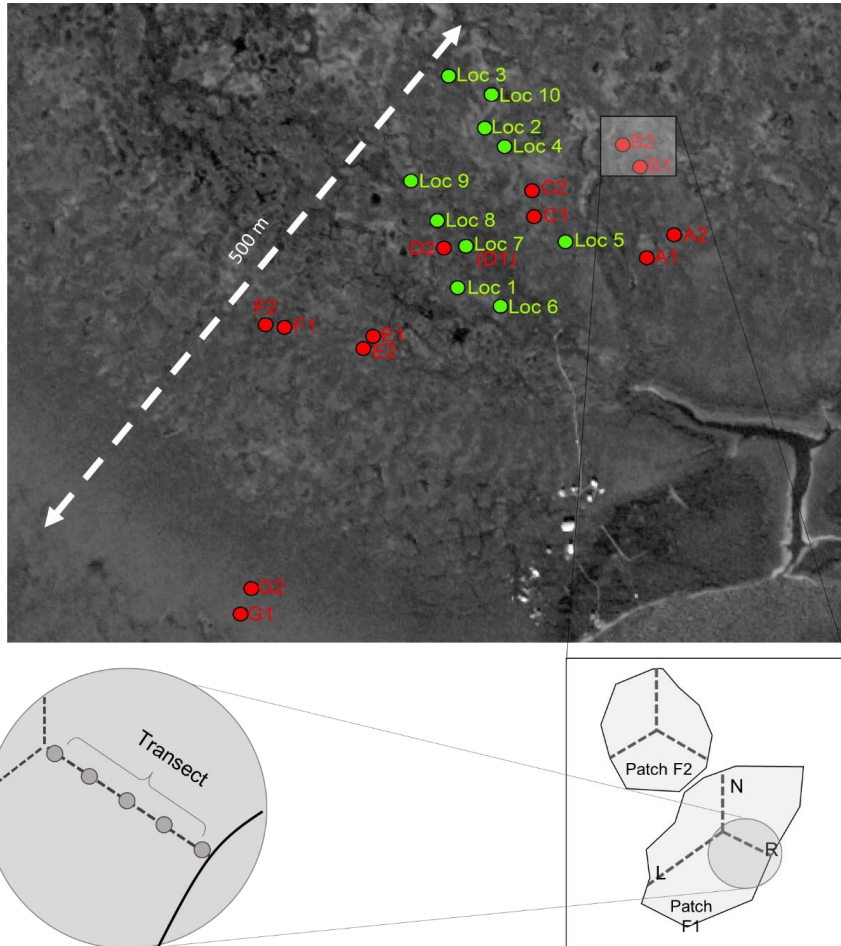
Fig. 4.2 *Map of the sampling locations at the focal Kytalyk research site. Green spots indicate the 10 patches (Loc1 to Loc5: Betula nana patches with a pond at the center; Loc 6 to Loc 10: B. nana patches without pond) where ramet samples for the dendrochronology study were collected in late July 2013; Red spots indicate the 14 patches where the leave samples for the microsatellite study were collected in July 2014 (A1, A2, B1, B2, C1, C2, D1, D2, E1, E2, F1, F2 were in the former lake bed, while G1 and G2 were at the Pleistocene ridge). In each patch, there were three transects (N, R and L; depicted in the square inset) with 5 spots per transect for sampling for the microsatellite study (depicted in the circular inset), and 3 spots per transect for the dendrochronology study)*

From each plant, 3 to 5 fresh leaves were collected and immediately stored using silica gel granules. The samples were kept at room temperature until arrival in the lab where they were stored at -20 °C.

DNA extraction and microsatellite analysis

First, the leaf material was crushed in liquid nitrogen using a Retch M400 grinding mill. Total DNA was then extracted using a PowerPlant® Pro DNA Isolation Kit (Mobio, 13400-50) based on the manufacturer's protocol. Samples were genotyped using six nuclear polymorphic microsatellite loci originally designed for Betula pendula (e.g., L1.10, L2.7, L13.1, L5.4, Lo21; see Table 4.1), after (Kulju et al., 2004) and *Betula pubescens* ssp. *Tortuosa* (e.g., Bo. G182, see Table 4.1, after (Truong et al., 2005).

Table 1 *Primer sequences and characteristics of the six microsatellite loci isolated from Betula nana, including locus name and GeneBank accession number (GenBank ref.), size of the sequenced allele (Size), motif in clonal allele (Repeat), PCR annealing temperature (Ta, °C), and MgCl$_2$(mM)*

Locus	GenBank ref.	Size (bp)	Repeat	Ta (ºC)	MgCl$_2$(mM)
L1.10	AF310856 †	152–206	$(AG)_4AA(AG)_{11}$	55	1.25
L2.7	AF310850	141–186	$(TC)_8(TA)_8(TG)_{11}TT(TG)_3$		
L5.4	AF310862 †	134–188	$(TC)_{26}$	50	1.25
L13.1	AF310871	93–108	$(CA)_3(GA)_{14}$		
L021	AF310877	184–192	$(CT)_{13}$		
Bo.G182	AY423617	120–160	$(TC)_{16}(AC)_5$	45	1.875

The PCR reactions were modified after (Kulju et al., 2004). The PCR reaction mixture (25 µL in total) contained 2 µL diluted DNA (5 ng/µL), 2.5 µL 10X PCR buffer, 0.5 µL MgCl2 (50mM), 1 µL BIOTAQ DNA polymerase (Bioline, BIO-21060), 0.5 µL Fluorescently labelled (Beckman, D2/D3/D4-PA) Forward primer (10µM, Sigma genosys, UK), 0.5 µL Reverse primer (10µM, Biolegio, NL), 1 µL dNTP's (Fisher scientific, NL) and 17.5 µL RNase-free water. PCR reactions were performed using the TProfessional thermocycler (Biometra). Shortly, the initial denaturation was 2 min at 94 °C, followed by 30-40 cycles (34 cycles for L1.10 and L13.1, 38 cycles for L2.7 and Bo. G182, 30 cycles for L5.4, and 40 cycles for Lo21) at 95 °C for 30 s, annealing at 62 °C for 30 s (L 5.4: 61 °C, L021: 56 °C), elongation at 72 ° C for 35 s and a final elongation at 72 ° C for 2 min. Amplification products were visualized on a Beckman CEQ 8000 capillary sequencer using 1 µL of PCR product and 27 µL Hidi formamide (Thermo Fischer scientific) and 0.35 µL DNA size standard kit 600 (Sciex). Bands were scored manually using Genemarker 1.95 (Softgenetics).

Micro-Checker software (version 2.2.3) was used to find potential genotyping errors such as microsatellite null alleles, stuttering and allele dropouts (Oosterhout et al., 2004). The result of the test suggested that the microsatellite Bo. G182 was probably a null allele, and thus that this micro-satellite might not efficiently reflect the genetic differentiation among samples. Accordingly, only data of the remaining 5 micro-satellites was used for the following analyses.

At last, vegetative ramets of different age classes belonging to 8 different clonal lineages were sampled to test the rate of somatic mutations. No genetic variation was observed between different ramets within a clonal lineage, assuming a low somatic mutation rate.

<u>Clonal identification and genetic diversity analysis</u>

Genetic diversity was estimated by the number of genotypes detected (*G*), proportion of distinguishable genotypes (*PD = G/N*) and Simpson's index of genotypic diversity ($D = 1 - \sum P_i^2$, P_i stands for the frequency of each allele for popluation, Ellstrand and Roose, 1987). A distinct multilocus genotype was considered a distinct genotype, while individuals with identical multilocus genotypes were identified as the same genotype (i.e., belonging to the clonal lineage). Moreover, estimates of genetic diversity of each population were obtained using GenAlex (version 6.5, Peakall and Smouse, 2012): mean number of alleles per locus (A), mean number of effective alleles per locus (Ae = $1/\sum p_i^2$), Shannon's diversity index (H = -1× (pi × Ln (pi)), observed heterozygosity (Ho) and Nei's unbiased expected heterozygosity (He =$1 - \sum p_i^2$). At last, pairwise's Fst values between different patches were calculated and plotted against geographic distance to test for genetic-by-distance isolation. The geographic distances were based on GPS coordinates taken in the fields. The correlation among these distances was tested by Pearson's tests in SPSS software (IBM SPSS Statistics for Windows, ver. 22.0; IBM Corp., Armonk, NY, USA). Finally, the analysis of molecular variance (AMOVA) framework (Meirmans, 2006) was used to calculate the genetic variation between and within patches.

4.3.3 Dendrochronological study on the age distribution of the B. nana patches

<u>Stem sampling strategy</u>

In late July 2012, 10 *B. nana* dominated shrub patches were selected in the former lake bed (Fig. 4.2). Five of these patches have ponds, which is presumably caused by local abrupt permafrost thaw. These two types of patches might have different histories of establishment and expansion. The history of these established *B. nana* shrubs is yet unknown, even though a few dendrochronological studies showed that that most of the living *B. nana* ramets (clonal individual) are no older than 60 years (Blok et al., 2011; Li et al., 2016). The mean area of the studied shrub patches is 412 ± 283 m2. Inside each patch, we set up three sub-transects from patch center to patch margin (Fig. 4.2). Along each transect sampling was conducted at three evenly distributed within-patch positions, i.e.Inner, Middle and Outer part, Fig. 4.2) with at least 50 cm distance between locations. At each location one *B. nana* ramet was randomly collected by excavating the ramet including root parts. In total 90 ramets (9 per patch) were collected for dendrochronological analyses.

From each ramet we obtained three, approximately 2-cm long stem sections: 1) from the shoot-root transition zone (located below soil surface), 2) the stem part located at the soil surface, and 3) the stem part just below the first branch (approximately 5 cm above the soil surface). The samples were preserved in an alcohol-glycerin mixture to prevent infection by fungi.

The soil abiotic conditions were assessed by measuring relative surface elevation, actual thawing depth and soil moisture content. The surface elevation and volumetric soil moisture content of each sampling location was measured in late July 2012 by using an optical levelling instrument (Kompensator-Nivellier NI 025, Jena, Germany) and a theta soil moisture probe (Delta-T Device, UK). At each patch, the elevation of the highest sampling position was considered as the 0 m reference.

The age of the ramet was determined by following the serial-sectioning approach (Gärtner et al., 2014) which implies detecting and measuring the shrub rings in the three stem sections taken along the ramet and cross-dating of the resulting ring-width series to verify correct ring detection and age determination. First, transversal thin sections (c. 20 μm thick) were prepared from the three stem samples per ramet by using a GSL1 microtome (Gärtner et al., 2014). We used astra blue/safranin mixture to stain these thin sections to enhance color contrast between lignified and undignified wood tissues. After dehydration, the thin sections were fastened on glass slides.

Shrub-ring detection and measurement was done from the micro-thin sections under a Leica MS5 microscope coupled to a LINTAB tree ring measurement device with the software WinTSAP (RINNETCH, Heidelberg, Germany). Per stem sample we measured ring width with a resolution of 0.01 mm along four radii as ring formation around the stem was eccentric as a consequence of the occurrence of (partially) missing rings; a common phenomenon in Arctic shrubs (Myers-Smith et al., 2015). The resulting shrub-ring series were subsequently visually and statistically (program WinTSAP, t-test, Pearson corr.) compared, i.e. crossdated, within and between stem disks. After successful crossdating, mean ring-width series were calculated for each stem disk and each ramet. The mean series for the 90 ramets where then crossdated (program COFECHA, Holmes, 1983) to verify consistency in ring-width patterns which allows for correct age determination.

Shrub-age distribution within patches and micro-topography

From the crossdated ring-width series, the year of establishment and hence the age of the 90 ramets can be determined, which provides a general idea on the time of establishment of the 10 shrub patches. The age distribution of the nine shrubs within a patch was moreover used to reconstruct patterns in patch expansion across time. Based on the age of the shrubs along the 30 transects in the 10 analyzed shrub patches it was tested whether the ramet ages followed the assumption that the oldest ramets were located at the patch center while the ramets at the middle and outer positions were consecutively younger. As we assumed that the two patch types (with/without ponds) might have different histories of establishment, we analyzed the age patterns of these two patch types by using a linear mixed model including within-patch position, patch type (with or without pond) and the interaction of these two factors as fixed factors, and patch code as random factor in the analysis. In addition to ramet age, we also tested for significant differences in soil moisture, relative elevation and active layer thickness (ALT) by using the same linear mixed model setup. All tests were ran using SPSS (IBM SPSS Statistics for Windows, ver. 22.0; IBM Corp., Armonk, NY, USA).

4.4 Results

4.4.1 Genetic diversity and geographic structuring of genetic variation

Only 2 out of 220 samples from all the 15 patches were identified as belonging to the same clonal lineage at the two study sites by the molecular analysis (Kytalyk site and Kodak site, patch A1: N = 15, G = 14, PD = 0.93, D = 0.98, Table 4.2). The two genetically identical ramets were neighboring plants and only around 2 m apart. In addition, relatively high levels of heterozygosity were observed (Table 2) with levels for observed heterozygosity (H_o) and expected heterozygosity (H_e)

higher than 0.7.

Table 4.2 *Genotypic diversity with different based on microsatellite data of Betula nana collected in Kytalyk station (A1 to G2), and Kodak station (42km away from Kytalyk station) in northeast Siberian tundra*

Pop	N	G	PD	D	A	Ae	H	Ho	He
A1	15	14	0.93	0.98	10.0	6.320	1.873	0.689	0.758
A2	15	15	1.00	1.00	10.0	5.882	1.877	0.786	0.767
B1	15	15	1.00	1.00	9.6	6.276	1.865	0.733	0.700
B2	15	15	1.00	1.00	9.8	5.683	1.860	0.701	0.756
C1	15	15	1.00	1.00	9.8	6.144	1.931	0.716	0.786
C2	15	15	1.00	1.00	10.4	5.644	1.900	0.733	0.773
D1	15	15	1.00	1.00	9.4	5.695	1.758	0.692	0.714
D2	15	15	1.00	1.00	11.2	7.059	2.080	0.796	0.818
E1	15	15	1.00	1.00	10.2	6.184	1.941	0.773	0.778
E2	15	15	1.00	1.00	11.6	7.442	2.068	0.710	0.801
F1	15	15	1.00	1.00	10.8	6.914	2.057	0.773	0.829
F2	15	15	1.00	1.00	10.0	6.657	1.977	0.686	0.812
G1	15	15	1.00	1.00	10.0	6.610	1.963	0.769	0.796
G2	15	15	1.00	1.00	10.6	6.525	2.026	0.827	0.814
H	10	10	1.00	1.00	9.2	6.311	1.980	0.740	0.822

N = sample size per population, *G* = number of multilocus genotypes, *PD* = ratio of distinguishable ramets (*G/N*), *D* = Simpson's index of genotypic diversity corrected for finite same size, *A* = mean number of alleles per locus. A_e = mean number of effective alleles ($1/\sum p_i^2$), *H* = Shannon's diversity index ($-1 \times (p_i \times \text{Ln}\,(p_i))$), H_o = observed heterozygosity and H_e = expected heterozygosity ($1 - \sum p_i^2$). The p_i stands for the frequency of each allele for the population.

The correlation between pair-wise genetic (F_{st} values) and geographic distance (km) was significant ($F = 0.881$, $p < 0.001$, Fig. 4.3a) when both the populations of the Kytalyk station and that of the Kodak station were included. However when only patches of the Kytalyk site were included no significant isolation-by-distance was observed ($F = 0.070$, $p = 0.513$, Fig. 4.3b). This suggests that the *B. nana* population at the Kodak station differed significantly from the populations in the focal site
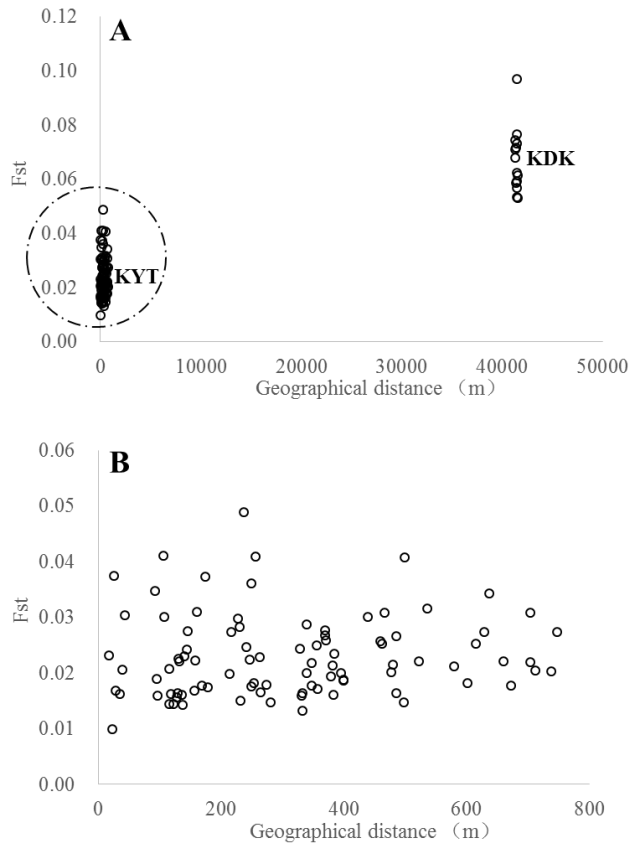
Fig. 4.3 *Relationships between pairwise genetic (F_{st}) value and geographic distance (m) for (a) all 15 patches, consisting of 14 populations (A1, A2, B1, B2, C1, C2, D1, D2, E1, E2, F1, F2, G1, G2 from Kytalyk station (KYT), and 1 population H from Kodak station (KDK) 42 km away from Kytalyk station), and (b) only the 14 populations from Kytalyk station(KYT).*

Only a small proportion of the genetic variation could be explained by the between and within patch structures; among patch and within patch constituted respectively 2% and 12% of the total genetic variation (AMOVA, Table 4.3). The mean F_{is} and F_{it} values were 0.180 and 0.189 respectively indicating only minor deficiencies of heterozygotes with patches. A low fixation index (F_{st} = 0.012) was found, indicating that patches were genetically not differentiated and suggesting high levels of gene flow.

Table 4.3 *Results of the Analysis of molecular variance (AMOVA) for the microsatellite DNA variance. A population stands for the samples from a single patch. Only the samples from the Kytalyk site were used in the tests.*

Source of Variation	df	SS	MS	Est. Var.	Percentage (%)
Among patch	14	49.481	3.534	0.042	2
within patch	205	470.867	2.297	0.254	12
Error (unexplained variance)	220	393.500	1.789	1.789	86
Total	439	913.848		2.085	100

df = degrees of freedom; SS = sum of squared deviation; MS = Mean Squares; Est. Var. = estimated variance.

4.4.2 Age patterns and micro-topography

The age of the 90 ramets sampled for dendrochronology was 36±14 yrs. Only 6 ramets were younger than 20 yrs, and 5 ramets were older than 60 yrs. Within-patch position and patch type did not significantly affect the age of *B. nana* (within-patch position: $F = 0.560$, $p = 0.574$, patch type: $F = 0.039$, $p = 0.845$, with-patch position × patch type: $F = 1.101$, $p = 0.337$). Only 2 out of the 30 within-patch transects showed the age pattern of oldest in the inner and youngest in the outer position.

While the ages of the *B. nana* samples were rather homogenous at the different within-patch positions of both patch types (Fig. 4.4a), the abiotic factors in the two different patch types showed distinctive patterns. Although thawing depth was not dependent on within-patch position or patch type (Fig. 4.4a), relative surface elevation and soil moisture did differ among the within-patch positions and/or patch type (Fig. 4.4c,d). Volumetric soil moisture was significantly higher at the inner position of the patches with pond, which had the lowest elevation as well. Meanwhile, the relative elevations at the outer positions of both plot types were significantly lower than those of the mid positions.
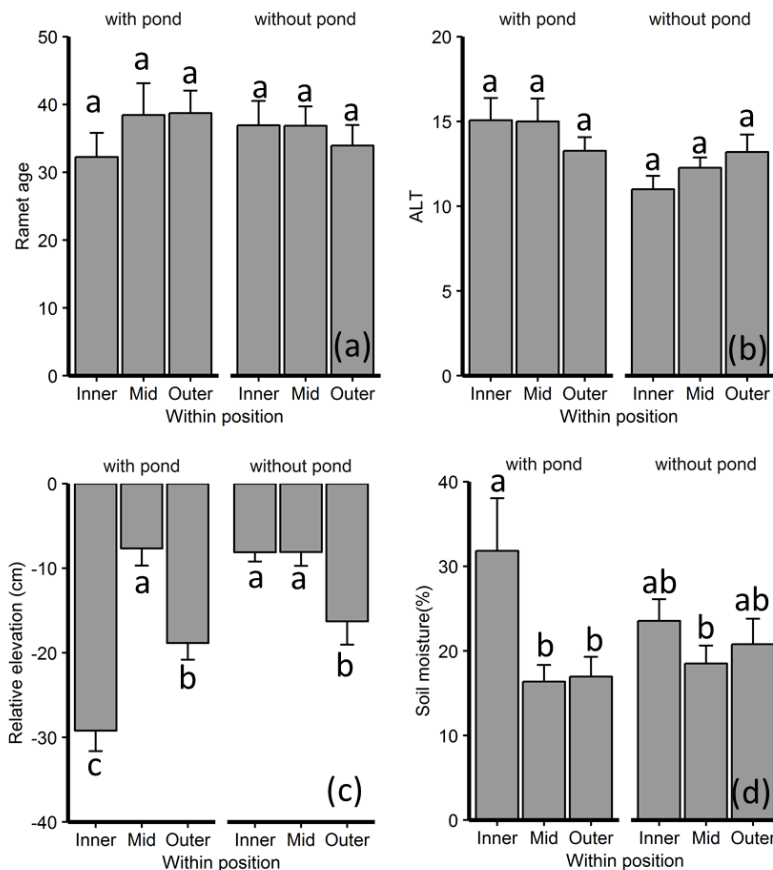
Fig. 4.4 *Age of the B. nana ramets (a), active layer thickness (ALT, b), relative surface elevation (c) and volumetric soil moisture content at 5 cm depth (d) of the different within-patch positions (inner, middle and outer) along the sub-transects in two different patch types (with ponds and without ponds). Different letters (a, b, c) represent significant differences among the within-patch positions. Data were mean values ± s.e.m.*

4.5 Discussion

4.5.1 Frequent sexual reproduction of B. nana shrubs

Our results challenge the prevailing but seldom tested consensus that sexual reproduction of perennial plants such as *B. nana* shrub is poor in the Arctic tundra (Billings and Mooney, 1968; Bliss, 1971; Callaghan and Collins, 1976; Bell and Bliss, 1980; Moulton and Gough, 2015). This study provided little evidence for dominance of vegetative recruitment of *B. nana* in the lowland tundra. Instead, the high genetic diversity of *B. nana* suggests sexual reproduction as a prevailing mechanism of reproduction of *B. nana*, which is in line with the findings of Douhovnikoff *et al.* (2012) on Salix spp. shrubs in Alaskan tundra. While high genotypic variance can also be found in

predominantly clonal populations (Ellstrand and Roose, 1987), due to sprouting of young *B. nana* ramets from centuries-old root remnants (Groot et al., 1997), our findings suggest that a different scenario is at work in the plants that we sampled. The dendrochronology showed that most of the *B. nana* ramet samples in this study were young and between 20-40 yrs old, which is in line with Blok *et al.* (2011) who showed a similar age distribution of *B. nana* ramets at the same site.

Nevertheless, this result does not mean that the vegetative reproduction of *B. nana* is rare at the study site. Horizontal belowground roots/stems of *B. nana* shrubs form a spider-net like layer covering every *B. nana* dominated patch (Appendices 1, *Li.*, personal observations), implying widespread clonal reproduction of *B. nana*. The contradiction between the field observation and the results of the microsatellite analysis is probably explained by the feature of the vegetative production of *B. nana*. As the mean distance between the neighboring spots for the sampling of the microsatellite study within each patch is around 2 m, we therefore suggest that most of the *B. nana* ramets reproduced through prostrate roots/stems are less than 2 m away from their parent plants.

Furthermore, the lack of genetic differentiation between different patches within Kytalyk site in combination with relatively high levels of genetic variation suggest high levels of gene flow between the patches in a small landscape scale (maximum distances between two patches are 500 m). *B. nana* shrubs produce large amounts of wind-dispersed pollens and seeds (Groot et al., 1997), genetic exchange within the focal study area (Kytalyk site) may therefore not come unexpected, providing ample favorable conditions for germination and establishment. A seed bank study on *B. nana* in an Arctic tundra by Molau and Larsson (2000) revealed that *B. nana* seeds in the Arctic tundra were sometimes transported to sites that were at least a few hundreds of meters apart. This finding also implied sexual reproduction of *B. nana* in Arctic tundra regions. Distinctive genetic differentiation was however found at larger geographic distances, between the *B. nana* populations from Kytalyk site and the population from Kodak site around 40 km away (Fig. 4.4a), indicating limited gene flow over larger distances. We therefore deduce that pollen and/or seeds are transported freely among different populations at relatively small geographic scales. Accordingly, contrary to expectation, sexual reproduction of *B. nana* shrubs at research site is prevalent, while the clonal recruitment is mainly limited to a small range around the parent plant.

4.5.2 Establishment and expansion of the B. nana patches in a lowland tundra

We found no evidence supporting the hypothesis that *B. nana* patches at the focal site had progressively enlarged from the patch center to the surrounding areas by clonal recruitment over time. The result of the dendrochronological analysis did not show any obvious age gradients from the patch center to the edge. Moreover, we found that ages of the *B. nana* within-patch positions were not significantly different, implying that these *B. nana* patches had not experienced a simple radial expansion as we assumed. Sexual reproduction is thought to be an important mechanism for the establishment and expansion of *B. nana* in the study site.

Successful sexual reproduction of the *B. nana* largely depends on the climate condition. Harsh abiotic stress such as short growing season with cold temperature often inhibits the sexual reproduction of the deciduous shrubs in the Arctic tundra. However, our focal site is located in Subzone 4, according to the classification of Arctic tundra by Walker (2000), which is the warmest

part of the Arctic Tundra Zone with mean July temperatures of 9-12 °C. The mean July temperature (10 °C between 1981-2010) at the study site is 4-5 °C higher than the mean July temperature of the northernmost bioclimatic zone where *B. nana* occurs. (Alsos et al., 2007), which is assumed to be suitable for seed production (Groot et al., 1997). Thus, summer temperature is probably not a critical barrier hampering the sexual reproduction of *B. nana*, in spite of its large year-to-year fluctuation (Chapter 5).

Apart from seed production, the survival rate of the *B. nana* seedlings is also critical. Drought in summer is considered as the most important factor resulting in high mortality of shrub seedlings in the Arctic tundra (Gartner et al., 1983). Like the summer temperature, summer precipitation at the study site varies dramatically over years, which might strongly affect the survival of the *B. nana* seedlings. In the study by Li et al. (2016) on the same dendrochronological samples, the temporal distribution of the *B. nana* ramets was significantly related to summer precipitation, and most ramets established in the periods with high summer precipitation around 1970 and in the 1980s. It is therefore likely that the success of the sexual reproduction largely depends on the amount of summer precipitation.

Following the above, we predict that limitation for sexual reproduction will be further relieved under future climatic conditions. Indeed future climate change scenarios predict rising temperature and increased summer precipitation in the Arctic tundra, which may lead to enhanced expansion of *B. nana* via sexual reproduction. Whereas climate conditions are critical to the sexual reproduction of the *B. nana* shrubs, we did not find any significant connection between micro-topographic and the age distribution of the *B. nana* shrubs. One factor that needs consideration is the ponds inside some *B. nana* patches, which are probably caused by abrupt small scale permafrost collapse. Inside the subsided pond, the soil moisture is significantly higher compared to other locations within the same patch. Although dead plants were rare in the selected patches, mortality of *B. nana* plants has been observed in many pond areas resulting from soil subsidence due to local thawing of ice-rich permafrost, since *B. nana* cannot tolerate waterlogged environments. The study by Jorgenson et al. (2015) claimed that with continuous warming in the future, the chance of the abrupt permafrost collapse in the Arctic tundra will increase. Therefore, it remains difficult to predict future vegetation shifts in Arctic tundra areas which are vulnerable to thermokarst.

4.6 Conclusion

This study is one of the first researches focusing on the reproduction mode of *B. nana* shrub using molecular tools. The results suggest that sexual reproduction of *B. nana* in the Arctic tundra is largely underestimated, and sexual reproduction likely plays an important role in the expansion of the *B. nana* patches. The findings of our study are in line with other studies (Honnay and Jacquemyn, 2008; Steltzer et al., 2008; Douhovnikoff et al., 2010) suggesting that sexual reproduction of vascular plants in the Arctic tundra commonly occurs, more than was previously considered. The results of the dendrochronological analyses further suggest that sexual reproduction, which is largely affected by summer precipitation and temperature, might play an important role in the establishment of present *B. nana* shrubs.

4.7 Acknowledgements

4.8 Appendices

Fig. 4.A1 One complete B. nana plant with four ramets (white arrows) and belowground roots/stems excavated from one B. nana dominated patch at the study site.

Chapter 5: The role of summer precipitation and summer temperature in establishment and growth of dwarf shrub *Betula nana* in Northeast Siberian tundra

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5.1 Abstract

It is widely believed that deciduous tundra-shrub dominance is increasing in the pan-Arctic region, mainly due to rising temperature. We sampled dwarf birch (*Betula nana* L.) at a Northeastern Siberian tundra site and used dendrochronological methods to explore the relationship between climatic variables and local shrub dominance. We found that establishment of shrub ramets was positively related to summer precipitation which implies that the current high dominance of *B. nana* at our study site could be related to high summer precipitation in the period from 1960 to 1990. The results confirmed that early-summer temperature is most influential to annual growth rates of *B. nana*. In addition, summer precipitation stimulated shrub growth in years with warm summers, suggesting that *B. nana* growth may be co-limited by summer moisture supply. The dual controlling role of temperature and summer precipitation on *B. nana* growth and establishment is important to predict future climate-driven vegetation dynamics in the Arctic tundra.

Key words: global warming, Arctic, shrub dominance, *Betula nana* L., dendrochronology, summer precipitation

5.2 Introduction

Global climate change has markedly affected the ecosystems on Earth (ACIA 2005; IPCC 2013). Compared to other regions, the Arctic region is experiencing dramatic air temperature increase and more extreme precipitation events (Hinzman et al. 2005; McGuire et al. 2006). During the last four decades surface air temperature increased on average 0.4 ˚C per decade over the Arctic (Anisimov et al. 2007).

As a consequence, local vegetation composition has changed in the North American and Scandinavian Arctic (ACIA 2005). Especially deciduous shrubs have been observed to increase in Arctic tundra. Experimental studies in tundra ecosystems (Bret-Harte et al. 2008; Mack et al. 2004; Nowinski et al. 2010; Shaver et al. 2001; Walker et al. 2006) suggested that deciduous shrubs, especially *Betula nana* L. (dwarf birch), benefit from the increase in temperature, thereby becoming more dominant in the ecosystem. In the last 50 years shrub-patch expansion was apparent at different sites throughout Alaska (Sturm et al. 2001; Tape et al. 2006), Canada (Hudson and Henry 2009) and other Arctic tundra regions (Frost and Epstein 2014; Hudson and Henry 2009; Myers-Smith et al. 2011; Myneni et al. 1997; Stow et al. 2004). The observed shrub expansion could reduce tundra albedo, leading to additional regional warming (Blok et al. 2011b; Juszak et al. 2014; Loranty et al. 2011; Sturm et al. 2005).

Field experiments, aerial photographs and remotely-sensed data provided a general but coarse impression of tundra "greening" explained as shrub expansion during the last decades (Sturm et al. 2001). However, measurement errors caused by instrumentation drift, atmospheric effects (Stow et al. 2004) and low image resolution (Tape et al. 2006) limit the assessment of fine-scale shrub cover dynamics. Meanwhile, the time spans of most field-warming experiments are too short to unravel the covariation of shrub growth and local climate fluctuations (Bret-Harte et al. 2008).

Dendrochronology is a useful tool to explore the long-term relationship between climate and shrub growth and expansion (Bär et al. 2008; Myers-Smith et al. 2011; Myers-Smith et al. 2015b). Woody

plants living under extreme conditions, such as shrubs in the Arctic, usually respond sensitively to climatic variability in their vertical and radial growth (Bradley and Jones 1992; Mäkinen et al. 2003; Myers-Smith et al. 2015a). Compared to trees, application of dendrochronology to Arctic deciduous shrubs is challenging mainly related to low growth rates and frequently missing rings (Woodcock and Bradley 1994) but also due to irregular wood formation along and around the main stem (Schweingruber et al. 2011; Schweingruber et al. 2013).

Similar to other woody species in the Arctic tundra, *B. nana* forms exceptionally narrow annual tree rings (Groot et al. 1997; Hollesen et al. 2015; Meinardus et al. 2011). The average ring width of *B. nana* collected by Miller (1975) was merely 130 μm. Dendrochronological analyses of such material requires application of serial sectioning, i.e. studying multiple samples along the main stem to account for frequently occurring missing rings, specifically in outer stem parts (Wilmking et al. 2012). Despite being a common circum-Arctic species that seems responsive to climate warming (Hollesen et al. 2015; Shaver et al. 2001; Wahren et al. 2005), *B. nana* has only recently been used for dendrochronological studies (Blok et al. 2011a; Meinardus et al. 2011; Miller 1975).

As temperature determines the length of the growing season in Arctic woody species (Berner et al. 2013; Hudson and Henry 2009; Walker et al. 2006), we assume that radial growth of *B. nana* at our site in the Northeastern Siberian tundra is related to summer temperature. Pop et al. (2000) found that the bud break of *B. nana* is sensitive to spring air temperature and snowmelt. Since the growing season in the Arctic tundra is extremely short, an earlier start of the growing season usually results in increased growth. Precipitation may be crucial as well as neither waterlogged areas nor dry soil conditions favor *B. nana* growth (Groot et al. 1997). Precipitation could provide the main water supply for *B. nana* during the growing season, particularly when the shrubs grow on relatively well-drained soil or when the climate is dry. However, the growth of deciduous shrubs could also be restricted by extremely high summer precipitation due to anaerobic soil conditions that might develop (Lloyd et al. 2003). Aside from the rainfall in summer, winter precipitation (snow) can also affect shrub growth (Blok et al. 2015), as deeper snow cover in winter provides better insulation, which leads to higher turnover in soil organic matter, which is hypothesized to benefit shrub growth during the subsequent growing season (Sturm et al. 2001). The primary objectives of this study were to identify the main climate factors that determine annual growth and establishment of *B. nana*, a common Arctic deciduous shrub species.

5.3 Material and methods

5.3.1 Site description and climate data

We collected samples of *B. nana* from Kytalyk Nature Reserve (70˚49'N, 147˚28'E), located in the Indigirka lowlands in northeast Siberia, Russian Federation (Fig. 5.1). The whole study area is underlain by continuous permafrost and the sampling locations were in the former bed of a drained thermokarst lake. The local vegetation type is defined as G4 (tussock-sedge, dwarf-shrub, moss tundra) at the circumpolar Arctic Vegetation Map (Walker et al. 2005). *B. nana* is the most common deciduous shrub species and covers approximately 20% of the land in the area (Blok et al. 2011a). *B. nana* ramets (vegetative clone) mainly occupy the palsas (permafrost hummocks), while *Sphagnum* mosses and *Eriophorum* sedges dominate the waterlogged depressions. Although *B.*

nana shrubs produce seeds, they mainly depend on clonal growth for reproduction (Groot et al. 1997). Since the density of large herbivores is extremely low at the site, their impact on shrub growth is assumed to be minimal.
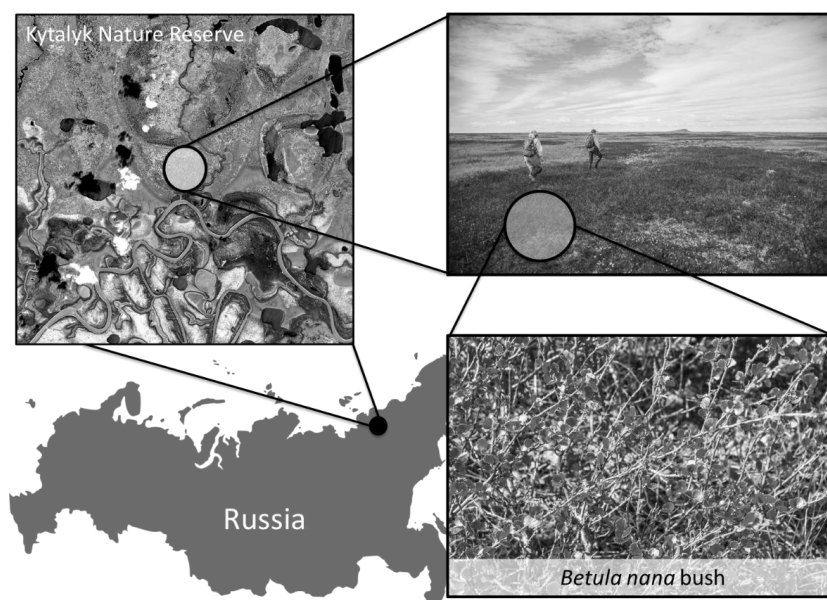
Fig. 5.1 *The map of our research site (Kytalyk Nature Reserve, Russia Federation)*

Long-term meteorological data (daily average temperature, daily cumulative precipitation and snow depth) since 1945 are available, recorded by the closest meteorological station (WMO station no. 21946 in Chokurdakh), 30 km south of the study site (http://climexp.knmi.nl/).The mean annual temperature (1981-2010) at Chokurdakh is -13.4 °C and the mean annual precipitation is 196 mm (1981-2010) (Nauta et al. 2015). The mean July temperature of the same period is 10.3 °C. The mean temperature in January is -34.0 °C. Summer is defined as ranging from June to August, as the snow-free period usually lasts from the beginning of June until the end of August accordingly. For each year, the start of the growing season and length of the growing season was calculated using these Chokurdakh climate data.

5.3.2 Sample collection and preparation

In total 90 *B. nana* ramets from 10 different *B. nana* dominated patches (approximate 173 m^2 in average) were systematically collected in July, 2012. All 10 patches were located at a former thermokarst lake bed (Fig. 5.1), and the distance among patches was at least 30 meters. In each patch, three transects (from center to patch edge) located at an angle of 120 degrees were established. Along each transect, three ramet samples (center, middle, outside) were collected (Fig. 5.A1).

The sampling strategy in general followed the requirements for applying the serial-sectioning technique to ensure detection and measurements of all tree rings at the stem base and along the

stem (Kolishchuk 1990) Individual ramet stems were cut just below the transition zone between root and stem (usually located a few centimeters below soil surface where the coarse roots started to appear) (Fig. 5.1). After measuring the total length of a ramet, three 2 to 3 cm thick disks were collected along the main growth axis: at the transition zone between root and stem (Disk α), at the soil surface (Disk β), and just below the first long branch (Disk γ). All stem sections were stored immediately in glycerin-alcohol-mixture (33% glycerin, 33% alcohol and 33% water in volume). The samples were transported to Wageningen University for further processing.

In the Lab, a 15 to 20 μm thick micro section was prepared from each disk of the three disks per ramet, using a GSL1 sledge microtome (Gärtner et al. 2014). For enhancing the contrast between different wood tissues which eases detection of ring boundaries, the sections were stained with a safranin/astra blue mixture. After five minutes staining, all the sections were dehydrated subsequently by using a 50%, 96%, and 99% alcohol. Dehydrated samples were then washed with Roti®-Clear liquid, and permanently imbedded in Roti®-Mount afterwards. All the slides were eventually fastened on a steel plate by using two button-shape magnets, and air dried for around 72 hours.

5.3.3 Tree-ring measurement, cross-dating and chronology development

Ring widths were measured directly from the slides along at least four radii using a combination of a Lintab digital positioning table and the TSAP-Win software (both Rinntech, Germany). Ring boundaries of *B. nana* are usually distinctly characterized by 1 to 3 tangential rows of flattened fibres (Fig. 5.2). Since ring formation was usually not concentric, some radii include narrow rings and (partially) missing rings. Correct dating of each measured ring as well as detection of missing rings is only possible through visual cross-dating of ring-width series and-if necessary-re-inspection of the rings on the slide. This process was successively applied, first within, and then between ramets. Within a ramet cross-dating starts on different radii of the same stem disk (α (upper main stem), β (soil surface) and γ (root/shoot transition), Fig. 5.2). After calculation of a mean ring-width series for each stem disk, mean ring-width series from disks taken at the three stem heights were compared to check whether the basal ring-width series (β) contains all rings and represents the annual variation in ring width across the ramet's lifetime. Visual cross-dating was statistically checked by applying the cross-dating quality control program COFECHA (Bunn 2008). If any missing ring was detected during the process, we manually added a ν narrow ring (10 μm) at that year in the ring-width series. As missing rings turned out to be more frequent in the radii of α disks in comparison to β disks (resp. 60% and 50% of all radii and on average 2 versus 1.5 missing rings per radius) but both disks showed no large difference in number of detected rings, disk β was used for further analyses.
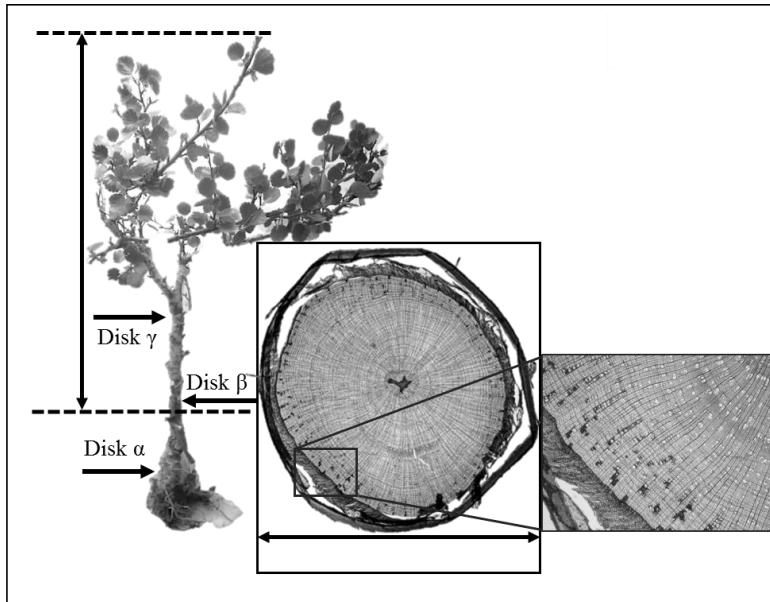
Fig. 5.2 *the photo of a thin section sample of Betula nana and the locations where the disks were collected (disk α, disk β, disk γ)*

The mean ring-width series derived from the β disks were finally used to (1) determine when the individual ramets established and (2) to calculate a site chronology. For the latter we used only individuals older than 30 years, as these are not only representing juvenile growth patterns, affected by initial competition-driven growth but mainly reflect environmental driven growth patterns of grown up, established shrubs. Before a site chronology was calculated from these older ramets, the individual ring-width series were standardized in ARSTAN v6.05 to eliminate size- and age-related growth trends. After standardisation the index series are averaged into a site chronology (program ARSTAN, Cook and Holmes 1986). The quality of this site chronology is checked by calculating the inter-series correlation (rbar) and the expressed signal (EPS) (Bunn al. 2008). Subsequently, the standardized ring-width index (RWI) was calculated. In addition, we compared the site chronology of *B. nana* in this study with the chronologies of *B. nana* and S*alix pulchra* Cham. In the study by Blok et al. (2011a). The RWI of *B. nana* (and *S. pulchra*) from both studies were compared as well.

5.3.4 Climate-growth analysis

Before analysis of climate-growth relationships, the normal distribution of each variable was checked by SPSS software (IBM SPSS Statistics for Windows, ver. 22.0; IBM Corp., Armonk, NY, USA). All variables showed a normal distribution, except some precipitation variables which were log-transformed to achieve or improve the normal distribution. Pearson's correlation coefficients (*r*) were calculated for the relationships between the RWI chronology and different climate factors: precipitation, temperature, snow depth, length of growing season (number of days with mean daily temperature over 5 ˚C per year, Briffa et al. 2008) and the start date of the growing season. The

start dates of the growing season during the period from 1952 to 2012 were calculated based on the equation developed by Pop et al. (2000). Parmentier et al. (2011) have calculated the start dates of the growing seasons of this region by using the same formula. In addition to precipitation, we also tested a drought index which combines precipitation and temperature data. Standardized Precipitation-Evapotranspiration Index (SPEI, Vicente-Serrano et al. 2010) data were obtained for the study site location from the global SPEI database v2.3 (sac.csic.es/spei/database.html, Vicente-Serrano et al. 2010). Since climatic conditions can also affect next year's radial growth (Buchwal et al. 2013), the Pearson's correlations for the relation between RWI and the climatic variables of the preceding year were included into the analyses.

5.3.5 Climate-establishment analyses

For each ramet sprouting year was based on the number of measured rings on the β disks (from soil surface). From the sprouting years of all 90 samples, we obtained the annual establishment numbers. In order to explore the relationship between climate change and shrub ramet establishment, we used the generalized linear model that takes the Poisson distribution of count data into account (in SPSS) to examine the relation between the ramet establishment number (dependent variable) and climate variables (covariate). As the year-to-year variation, particularly in precipitation, is very large and the exact ages of the samples can be underestimated due to undetected missing rings especially in very young individuals which are difficult to crossdate, we also used 5-year sums of establishment with 5-year averages of climate data, to examine relationships between establishment and climate factors.

5.4 Results

5.4.1 Age-related cambial growth and RWI chronology

B. nana showed a pronounced negative logarithmic radial growth trend with increasing age (R2 = 0.85) (Fig. 5.A2). The ring-width increment declined sharply, especially during the first 15 years (slope = .9.68 μm·yr^{-1}, R^2 =0.92), and this trend became much weaker in the later years (slope = -0.25 μm·yr^{-1}, R^2 = 0.19). The results from *B. nana* samples from Blok et al. (2011a) showed a similar result (Fig. 5.A2). In contrast to *B. nana*, the growth rate of *S. pulchra* showed a constant negative linear trend (slope = -2.68 μm·yr^{-1}, R2 =0.51) and ring widths were on average much larger (*B. nana*: 81.03 ± 38.91 μm, *S. pulchra*: 189.11 ± 61.59 μm, Fig. 5.A3). Based on these 53 *B. nana* individuals, a final *B. nana* chronology was established. The chronology of *B. nana* was characterized by a reasonable common signal represented by rbar (0.351) and EPS (0.933). In addition, we observed that the ramets samples, which have been collected on July, 20 in 2012, have final row(s) of flatted fibres (the ring boundaries of 2012).

5.4.2 Influence of climate factors on radial growth

Year-to-year variation in *B. nana* radial growth was significantly related to mean summer temperature (r = 0.47, p = 0.001, 1962-2011, Fig. 5.3), but correlations with other temperature-related variables (starting date and length of growing season and previous year's summer temperature) were not significant (p > 0.05, Table 1). The temperature in the period from June 16 till July 20, hereafter referred to as early summer, appeared most influential (Table 5.1, r = 0.727,

p < 0.001, *n* = 50 years). The late summer temperature (July 21-August 30) had no significant effect on the shrub radial growth (*p* > 0.05).
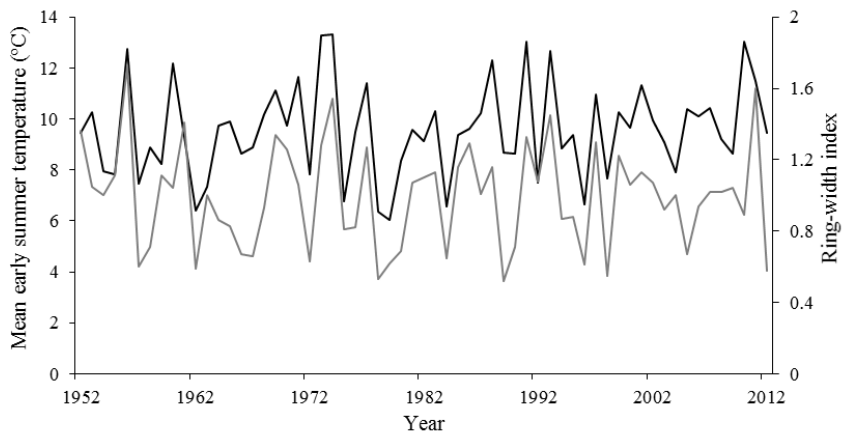
Fig. 5.3 *Ring-width Index of Betula nana (grey) and the mean early summer temperature (black, ˚C, 16th of June to 20th of July) from 1952-2012*

Table 5.1 *Pearson's correlation coefficient for relationships between annual ring-width index (RWI) of Betula nana and local climate variables (summer temperature (June-August), early summer temperature (June 16-July 20), precipitation (cumulative, June-August), mean snow depths (previous year's October-May) and growing days and standardized precipitation evapotranspiration index (SPEI) in summer) over the years 1962-2011 (n = 50 yrs), the years with warm summers (early summer temperature above median (9.58 °C), n = 25) and the years with cool summers (early summer temperature below median, n = 25).*

Climate variable	1962 – 2011					
	All years (n = 50 years)		Warm summers (n = 25 years)		Cool summers (n = 25 years)	
	Coeff.	P-value	Coeff.	P-value	Coeff.	P-valu
Temperature variables (ºC)						
Length of growing season	**0.289***	0.046	0.249	0.253	0.302	0.1
Start date of growing season	0.099	0.493	0.177	0.396	-0.062	0.7
Summer temperature	**0.496****	<0.001	0.052	0.813	0.296	0.1
Early summer temperature	**0.727****	<0.001	**0.468***	0.018	**0.524****	0.0
Previous year's summer temperature	-0.084	0.568	0.013	0.950	-0.001	0.9
Previous year's early summer temperature	0.042	0.772	0.339	0.098	-0.163	0.4
Precipitation variables (mm)						
Summer precipitation	0.136	0.348	**0.530****	0.006	-0.190	0.3
Previous year's summer precipitation	0.139	0.336	**0.451***	0.024	-0.124	0.5
Mean snow depth (cm)	-0.084	0.560	0.042	0.840	-0.243	0.2
SPEI						
SPEI Jun-Aug	0.107	0.458	**0.408***	0.043	-0.091	0.6
Prev. SPEI Jun-Aug	**0.286***	0.044	**0.445***	0.026	-0.097	0.6
SPEI previous year's Jun -Aug	0.208	0.146	**0.507***	0.010	-0.151	0.4

*The coefficients with * and ** represent a significant correlation between the RWI and the climatic variable (p < 0.05; p < 0.01).*

Precipitation also influenced radial growth patterns, but only in the years with a warm summer (Table 5.1). Annual radial growth rates were significantly correlated to both current-year and previous-year summer precipitation (Table 1) when the early summer temperature was above 9.58 °C (the median during the 50 years, 1962-2011). In the years with a below-median early summer temperature, only early summer temperature was significantly related to RWI and no other climatic variable (Table 5.1). The mean snow depth did not significantly correlate with radial growth (Table 5.1). The relationship between SPEI and *B. nana* RWI values were significant only in the years with warm summers (Table 5.1, r were between 0.41 and 0.51, $p < 0.05$). SPEI over the period previous-year June up to current-year August had the largest correlation coefficient ($r = 0.51$, $p = 0.010$, $n = 25$ yrs), with high SPEI values indicating wet climatic conditions, taking evapotranspiration into account, corresponding with high ring widths.

5.4.3 Influence of climate factors on ramet establishment

Most of the 90 collected *B. nana* ramets established 20 to 50 years before sampling, while only five successfully established in the last 20 years, and the 10 oldest ramets initiated growth before 1963. The increase in establishment over the 1950s and 1960s coincided with increasing summer precipitation and SPEI over the same period (Fig. 5.4). Similarly, the dip in establishment numbers in the late 1970s was related to negative summer SPEI values indicating dry climatic conditions (Fig. 5.4). Establishment numbers were low since the mid-1990s in a period of declining precipitation. The 5-year establishment numbers over the period 1951-2000 were significantly related to June-August SPEI and precipitation, but were not related to temperature or temperature-related variables (SPEI Jun-Aug: p = 0.003, AIC = 58.4; summer precipitation: p = 0.018, AIC = 61.6; early summer temperature: p = 0.143, AIC = 64.8).
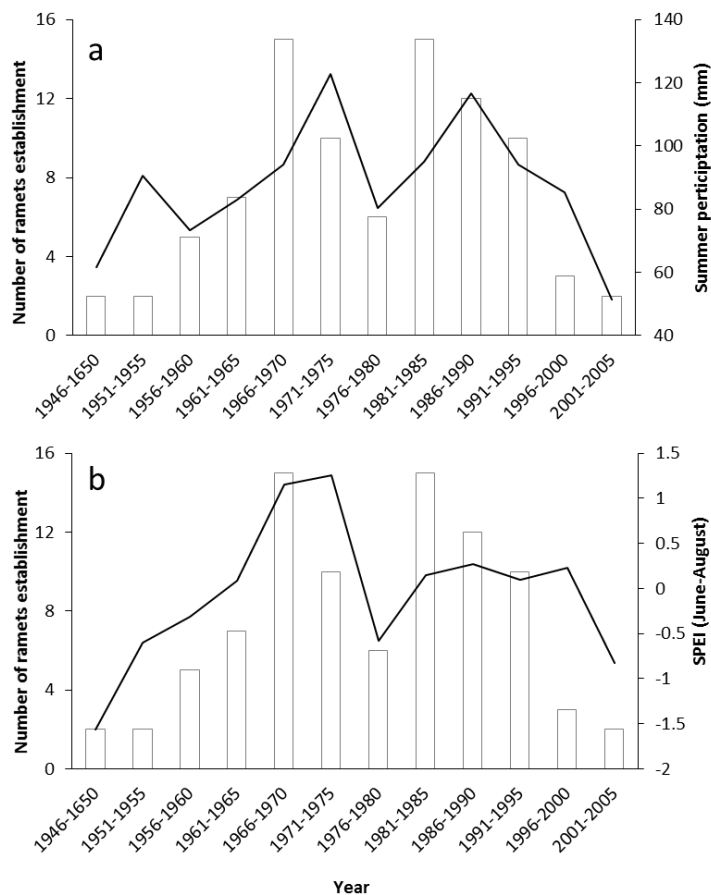
Fig. 5.4 The number of Betula nana ramets established (bar) and a) summer (June-August) precipitation (mm); b) standardized precipitation evapotranspiration index (SPEI) in summer (June-August) (line), both averaged per 5 years from 1946 to 2005.

5.5 Discussion

5.5.1 Climatic change and ramet establishment

In this study, a large number of *B. nana* samples were successfully cross-dated to build on a study by Blok et al. (2011) on this widely spread and hence important Arctic shrub species. Although ring detection is sometimes difficult due to eccentric growth and partly missing rings we succeeded in building a reliable *B. nana* site chronology with high rbar and EPS values.

The vertical growth of deciduous shrubs like *B. nana* can be largely restricted by local snow depth (Walker et al. 1997). Extremely low winter temperatures at our site can easily reach -30 ˚C. Without insulation by the snow cover, the shrub branches can hardly survive (Walker et al. 1997). Some studies in Scandinavia (Groot et al. 1997; Jonasson 1982) stated that snow cover largely influences the height of *B. nana* in tundra ecosystem. At our site, the maximum shrub height is approximately 30 cm (Fig. 5.1), which is more or less equal to the local average snow depth. In our dataset there was a positive relationship between shrub diameter at the soil surface and height ($r = 0.43$, $p <$ 0.01, $n = 90$, data not shown). Since the vertical and radial growth of woody species may be positively correlated (Hallinger et al. 2010), the height limitation controlled by snow depth could largely control the radial growth as well. This may explain why the ring-width growth of *B. nana* was initially fast, but declined quickly and stayed at very low levels after the first 15 years. Apart from the effect of snow depth, aging and competition may also influence the radial growth of *B. nana*. It is common in Arctic shrubs that after an initial juvenile stage, the ring widths continuously decline (Myers-Smith et al. 2015b; Schweingruber 1996). *Betula nana* may show a similar pattern here. Moreover, as shrub size increases, the competition for soil nutrients and light among the neighbouring individuals may be more intense. More research is needed to clarify the roles of snow depth and competition on the age-related cambial growth trends.

The possibility of cross-dating the ring-width series of all ramets enabled reconstruction of the dynamics of ramet establishment across shrub patches. Although the sampling strategy was not specifically designed for analysing ramet establishment in relation to climatic variations, we did obtain a dataset of 90 aged *B. nana* ramets which required measuring multiple radii in 3 stem sections per ramet and use crossdating to obtain reliable ages in this difficult species with partial missing rings. The sampling was not directed to sample the thicker ramets as the primary purpose was to investigate age distribution patterns within the shrub patches. We sampled along transects from the centre to the margin of the shrub patch. There was no relationship between age and position within the transects, and also the ages of the three centre positions, which were relatively close to each other, were highly variable. This lack of an age pattern suggests that we probably sampled a random sample and not specific age cohorts. However, it was striking that the temporal distribution of the ramet establishment was not random. It is clear that most ramets established in periods with high summer precipitation around 1970 and in the 1980s, while the old and young (established before 1960 or after 1995) were rare, following the significant decline in summer precipitation since 1991 (slope = 4.56 mm ⋅yr^{-1}, $R^2 = 0.54$, $n = 20$ yrs: 1991-2010). This suggests that more ramets established when summer precipitation was high. Soil moisture supply in *B. nana* patches is controlled by summer precipitation and depth of permafrost thaw (Lloyd et al. 2003). As a shrub species with a highly plastic growing strategy (Bret-Harte et al. 2008), it is plausible that *B.*

nana shrubs prefer to sprout new ramets when soil moisture is adequate, at the cost of stem growth (both radial and vertical). This may also explain the relatively low correlation between the radial growth curves and the year-to-year summer precipitation in our study. The continuously decreasing summer precipitation during the last two decades could be responsible for the low number of young ramets, i.e. recently established ramets among all samples.

We assume that the bias of missing old *B. nana* samples or young ramets during collection is low. Like many Arctic shrubs, *B. nana* shrub ramets can easily survive longer than 50 years (Groot et al. 1997). Dead stems are well preserved for years at our sites, due to the low decomposition rates of recalcitrant low-quality woody material in the harsh climate. Nevertheless, we found very few dead *B. nana* ramet relics inside the patches. Furthermore, for the statistical analysis we excluded the periods in which the oldest and youngest ramets established as we cannot be sure that we missed young or old ramets subjectively.

The negative precipitation trend and associated low establishment together with a lack of a positive trend in annual ring width index could imply that there is no shrub expansion at our study site. Unfortunately, the lack of a long-term positive trend in ring width index does not necessarily mean that climate warming did not affect shrub radial growth. The dendrochronological methods, e.g. detrending, remove gradual changes over time, as they could be the result of ontogenic, e.g. age-related, trends and optimise the inter-annual variation for investigation of growth-limiting factors. Perhaps there was a declining trend because of aging compensated by a positive trend because of climate change; it is impossible to distinguish between the two. Support for no shrub expansion comes from satellite NDVI records showing no greening trend for the study region over 2000-2010 (Blok et al. 2015). Local permafrost degradation resulting in thaw pond development and drowning of shrubs could have prevented net shrub expansion (Nauta et al. 2015). The negative precipitation trend is not unique to the study area, but also occurred in some other locations particularly in the High Arctic (Urban et al. 2014). We used the KNMI Climate Change Atlas tool (http://climexp.knmi.nl/atlas) to generate a map of summer (JJA) precipitation trends over the years 1991-2015 for the Arctic land based on observations (CRU TS 3.22 dataset). The map (Fig. 5.A3) shows that the trends in summer precipitation are far from homogenous in the Arctic. In our study region, the Indigirka delta, the mean regression relative summer precipitation (1991-2015) is -50% per century and the similar patterns also exist in other Arctic regions, e.g. Lena delta, Canadian High Arctic (Fig. 5.A3), while some other areas show strong positive trends. The large variation of trends in summer precipitation in the Arctic further suggests that shrub expansion might not happen everywhere in the Arctic tundra. However, future precipitation is expected to show an increasing trend as the retreat of sea ice results in strongly increased evaporation and precipitation (Bintanja and Selten 2014).

5.5.2 Climatic change and shrub growth

Several experimental studies in the Arctic (Hobbie and Chapin 1998; Mack et al. 2004; Wahren et al. 2005; Walker et al. 2006) pointed out that the growth of *B. nana* was stimulated by increasing air temperature during the growing season. Most dendrochronological studies on the Arctic shrubs also indicated summer temperature as the most important factor for shrub growth through lengthening of the growing season (Hallinger et al. 2010). Myers-Smith et al. (2015a) stated that

the summer temperature variable is the most influential factor that affects the variation of Arctic shrub growth. Our study confirmed that year-to-year variations in summer temperature strongly influenced the annual radial growth rates and that the mean temperature of early summer (June 16- July 20) was a more important factor for the radial growth of *B. nana* than that of the late growing season (Blok et al. 2011a).

Evidence from both the sample measurement and the field observation supported this deduction that early summer temperature is more important than late summer temperature for the width of a ring formed in a given year. In most ramets collected on July, 20 in 2012 we observed that the final row(s) of flatted fibres were already formed indicating that the formation of the 2012-ring was almost completed by July 20 (Fig. 5.A4). This suggests radial growth of *B. nana* already ceased. Furthermore, the leaf colour of *B. nana* usually starts to turn from green to yellow and red at the beginning of August at our site (Fig. 5.A5), indicating the degradation of chlorophyll (Addicott and Lyon 1973) and lower photosynthesis activities. It further explains why the length of growing season (number of days with mean daily temperature over 5 ˚C per year) was not strongly correlated with the radial growth of *B. nana*. Although the mean temperature is usually above 5 ˚C until the beginning of September, from this research it seems that the radial growth of *B. nana* finishes approximately 3 to 4 weeks earlier, at both the stem base (disk α) and the upper part of the stem (disk β and disk γ, Fig. 5.1).

Summer precipitation not only stimulated the sprouting of *B. nana* ramets, but facilitated the annual radial growth rates of *B. nana* shrubs as well, but only during warm summers. While low radial growth rates occur when early-summer temperatures are low, relatively warm summers positively affect radial growth rates of *B. nana* provided that summer precipitation, either in the actual summer and/or the previous summer are above-average. Remarkable was the peak growth rate in 2011 which coincided with a warm and wet summer, whereas the preceding summer of 2010 was even warmer but also very dry, resulting in a rather modest radial growth (Fig. 5.A2). At a nearby treeline research site, 2011 was also an exceptional year with high photosynthetic rate (Liang et al. 2014). It was recently stated that apart from summer temperature, soil moisture is another important factor that largely affects the growth of shrubs in the Arctic (Myers-Smith et al. 2015a). They further suggest that the importance of soil moisture to the growth of Arctic shrubs increased during warm summers. The results of our study supports this statement, due to the positive correlation between soil moisture and summer precipitation in the study region (Liang et al. 2014).   .

The effect of previous year's summer precipitation on shrub growth is probably related to local soil nutrient supply. In the Arctic tundra, rapid growth of deciduous shrubs, for instance *B. nana* and S. pulchra, in the early growing season (late June to early July) is mainly supported by belowground nutrient storage instead of nutrient absorption at the time that the soil is still mostly frozen (Chapin 1980; Chapin et al. 1990), which is a common strategy among Arctic plants. Due to the extremely harsh winter environment (frozen soil and low temperature) in northeast Siberian tundra, the belowground nutrient accumulation mainly takes place during the previous growing season (Bilbrough et al. 2000). The soil nutrient absorption of the previous years is critical to the shrub growth of the next seasons. Higher soil moisture in the Arctic tundra might accelerate the permafrost thawing at *B. nana* dominated patches during the growing season by increasing soil

thermal conductivity. Six years of thaw depth measurements at the research site revealed that summer thaw depth was largest in 2011, which had exceptionally large summer precipitation (Nauta et al. 2015). The larger thaw depth might lead to a higher nutrient availability in deeper soil (Wahren et al. 2005). Dry soil conditions at the surface could reduce decomposition of organic matter, potentially reducing nutrient supply (Blok et al. 2015). Further studies should include investigations on soil nutrient availability. Since growth of deciduous shrubs like *B. nana* is highly sensitive to changes in soil nutrient availability (Marschner et al. 2007), inter-annual variations in *B. nana* growth may follow variations in soil nutrient availability (Shaver et al. 2001).
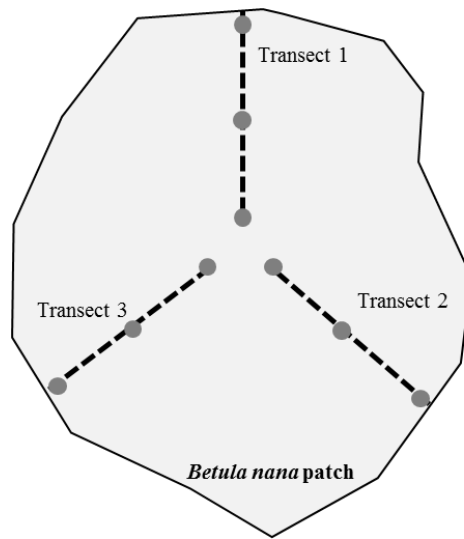
5.6 Conclusion

It has been widely shown that growth of deciduous shrubs like *B. nana* is responsive to changes in summer air temperature. This study builds on this result and further suggests that summer precipitation also influences growth rates, perhaps indirectly through soil nutrient availability, moreover summer precipitation seems to play an important role for shrub establishment. This implies that summer precipitation can facilitate the expansion of *B. nana* in Arctic tundra and that the influence of precipitation dynamics on vegetation composition changes in high-latitude ecosystems should not be ignored or underestimated.
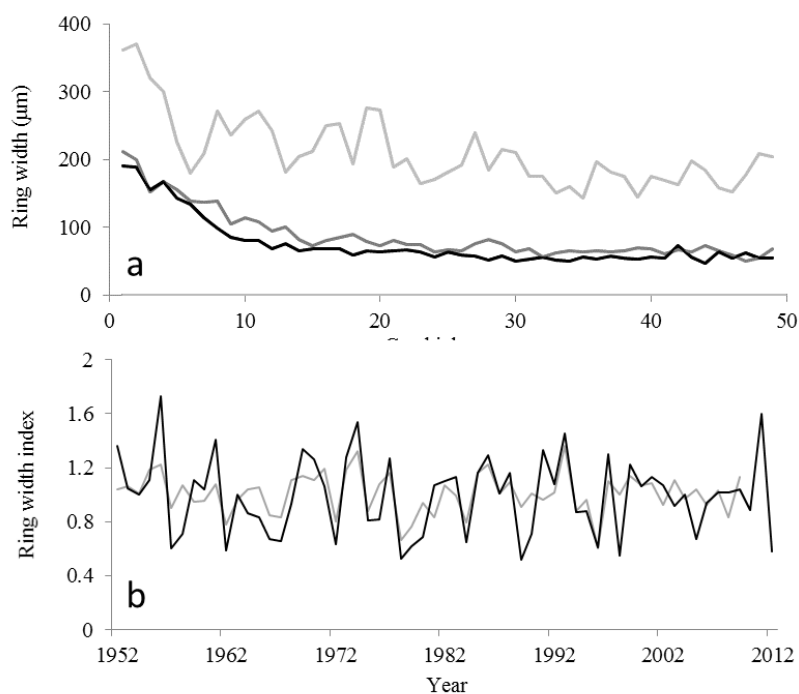
5.7 Acknowledgments

5.8 Appendices


Fig. 5.A1 *Strategy for collecting Betula nana ramet samples inside shrub patches. Three transects (Transect 1, 2 and 3) and three plots (inside, mid, outside) were defined. One sample was collected randomly at each plot.*

Fig. 5.A2 *a) 50-year long cambial growth curves of Betula nana (black, average of 53 samples), and both Salix pulchra (light grey, average of 19 samples) and Betula nana (dark grey, average of 19 samples) from the study of Blok et al (2011a)) ; b) the (RWI) chronology (1953-2012) of Betula nana (black) and that of Betula nana (grey, 1953-2009) from the study of Blok et al (2011a).*
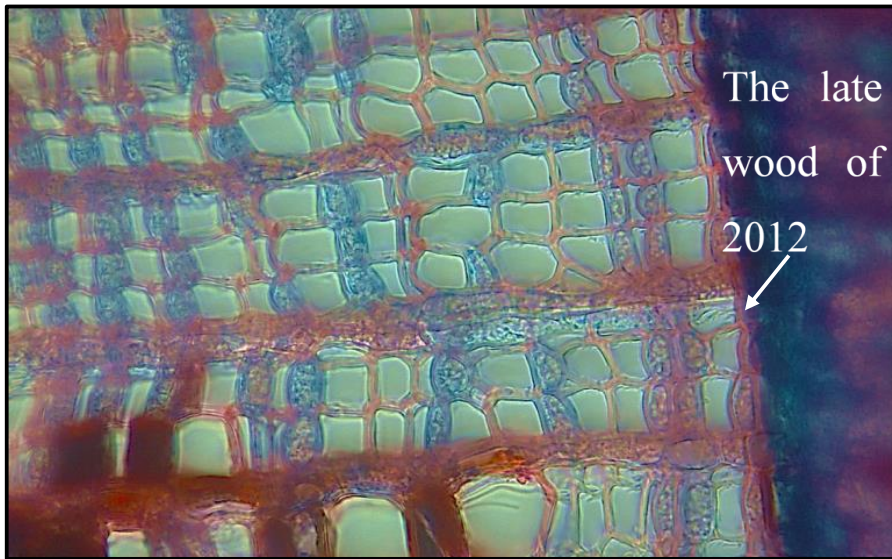
mean regression precipitation on time 1991-2015 Jun-Aug CRU TS 3.22

[mm/dy/100yr]

-2   -1   -0.5 -0.2 -0.1   0   0.1   0.2   0.5   1   2
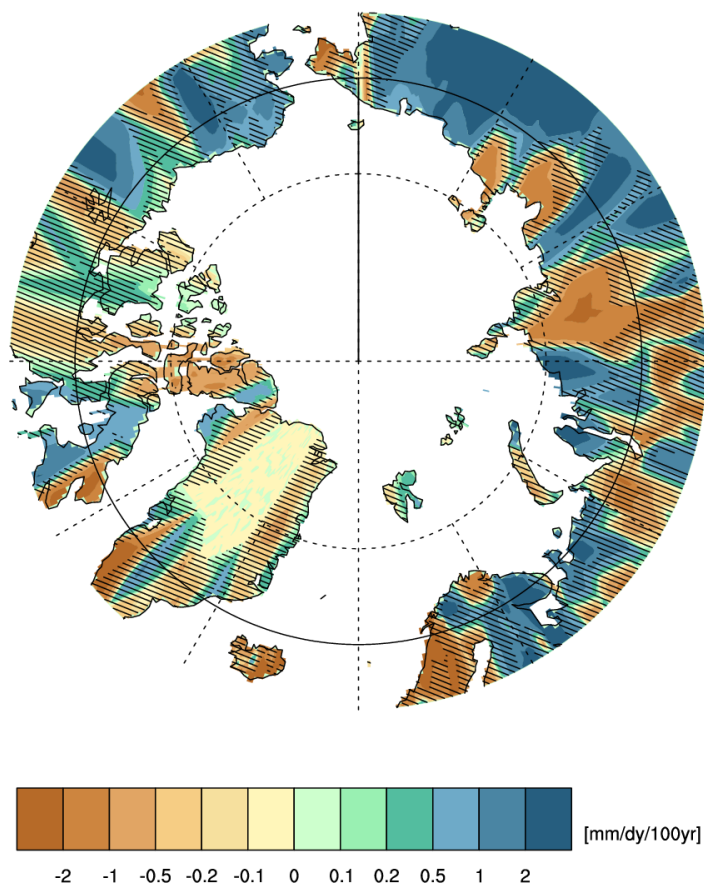
Fig. 5.A3 *The map of mean regression summer precipitation (June – August) of the Arctic between 1991 and 2015 based on the observation data (CRU TS 3.22 dataset) from the KNMI Climate Explorer (http://climexp.knmi.nl/atlas)*

Fig. 5.A4 *Starting late wood formation indicated by occurrence of flattened fiber cells in Betula nana sample collected on July 20, 2012*

Fig. 5.A5 *The picture of Betula nana leaves taken in 5th of August, 2012. The margins of the Betula nana leaves were turning red.*

Chapter 6: Synthesis

6.1 Introduction

Rapid changes in the climate of the high latitudes over the past century are believed to impact upon the vegetation composition of the Arctic tundra (ACIA, 2005; IPCC, 2013). Warming of the Arctic is considered to be responsible for the "greening" (expansion of woody plants) of the pan-Arctic region during recent decades (Stow et al., 2004; Tape et al., 2006; Myers-Smith et al., 2011; Myers-Smith et al., 2015). Experimental studies (Shaver et al., 2001; Mack et al., 2004; Walker et al., 2006; Bret-Harte et al., 2008; Nowinski et al., 2010) suggest that deciduous shrubs such as *Betula nana* L. are favoured under the warmer climate at the cost of mosses and other functional plant types. Pearson et al. (2013) predicted that the woody plant cover in the Arctic tundra, especially shrub cover, will increase by 52% in 2050 because of the continuous climate warming.

While the shrub expansion is widespread across the pan-Arctic region, the Arctic lowland tundra might have experienced more complicated vegetation shifts. The study by Jorgenson et al. (2006) suggested that abrupt small-scale permafrost collapse in the Arctic lowland tundra is norm, which may lead to the decline of shrub dominated patches (Lemke et al., 2007). As shrubs like *B. nana* can hardly tolerate waterlogged soil (Groot et al., 1997), extremely high soil moisture driven by the rapid permafrost thaw may lead to the decline of the shrub dominated areas and may favor other plant species, especially *Eriophorum* spp. sedges.

Vegetation composition is a pivotal non-climate factor that governs the soil surface energy balance, and therefore affects permafrost stability in ecosystems at high latitudes (van Wijk et al., 2004; Anisimov and Reneva, 2006). Shrub cover, for instance, is able to reduce surface albedo, increase evapotranspiration, and decrease active layer depth due to soil shading (Pearson et al., 2013). Shrub shading may (partially) compensate for the warming effect of increased air temperature on the soil, at least at the local scale (Jorgenson et al., 2010; Blok et al., 2011; Myers-Smith and Hik, 2013). The insulating capacity of the moss cover also contributes to stable permafrost conditions. In addition, vegetation shifts not only affect the permafrost stability, but also the greenhouse gas emission of the Arctic lowland tundra (Nauta et al., 2015), leading to significant shifts in the local carbon balance.

Although people are keen to talk about the vegetation shift in the Arctic lowland tundra and its influences on the environment, the vegetation succession in this ecosystem is yet largely unknown. Particularly in lowland tundra, which has ice-rich permafrost and is poorly drained, both shrub expansion and local shrub decline due to small-scale permafrost collapse can take place simultaneously. We used a combination of different methods, including dendrochronological analyses, genetic tools and field experiments, to explore the vegetation changes in a typical Arctic lowland tundra in the Northeastern Siberia. This chapter synthesizes the results found in these experiments and studies. First of all, I exhibit a general picture of the vegetation succession in the Arctic lowland tundra and discuss the interaction between the vegetation shift and the local permafrost dynamics. I then discuss the reproduction modes and the growth pattern of *B. nana*, one of the most common deciduous shrub species in this ecosystem, which is also one of the key

plant species with decisive impacts on vegetation succession, but also on permafrost stability. In addition, the effects of *Sphagnum* mosses on the carbon balance are analysed, since *Sphagnum* mosses might significantly affect greenhouse gas emissions. Finally, some suggestions and recommendations are provided for the future studies of vegetation dynamics in the Arctic lowland tundra.

6.2 Vegetation succession and permafrost dynamics in the Arctic lowland tundra

6.2.1 Vegetation succession cycle and permafrost degradation-recovery cycle

As the vegetation of the Arctic lowland tundra is sensitive to the hydrological conditions, different vegetation types occupy the areas with distinctive soil moisture levels, forming a mosaic-like landscape (Fig. 5.1). *B. nana* shrubs occupy the elevated areas with well-drained soils (Groot et al., 1997), while the *E. angustifolium* sedges prefer the water-logged areas or open water (Shaver and Chapin, 1991). However, this pattern is dynamic, largely due to the influences of both the vegetation composition and the permafrost. Teltewskoi et al. (2016) analyzed the proxies (pollen, macrofossils, testate amoebae, geochemistry and sediment properties) that had remained at the different depths of the permafrost at the same site. Their results clearly elucidate that dry periods dominated by shrubs and wet periods with sedges and mosses had alternatively appeared at the same location during the past 4000 years.

As the study period was limited, it was not possible to record the complete vegetation succession process using purely field observations. Here I combined the results of all four chapters to depict the whole process of vegetation succession in an Arctic lowland tundra of Northeastern Siberia. Based on the results of these studies, I suggest a vegetation succession loop in the Arctic lowland tundra which is accompanied by a permafrost degradation-recovery cycle (Fig. 6.1), while these two processes strongly interact with each other. In the vegetation succession loop, four stages dominated by distinctive vegetation types (*B. nana* shrubs, *Arctagrostis latifolia* (R.Br.) grass, *Eriophorum angustifolium* L. sedges, and *Sphagnum* mosses) are recognized.
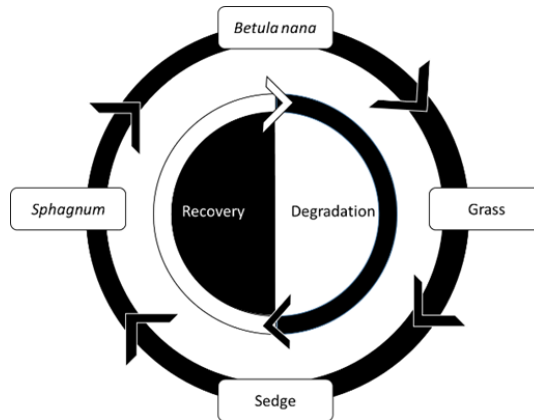
Fig. 6.1 *The conceptional graph showing the cycle of vegetation succession and circular route of permafrost dynamics in the Arctic moist tundra over time. The outer ring shows the circle of vegetation succession, including four distinctive stages (Betula nana dominated stage, grass dominate stage, sedge dominated stage and the Sphagnum dominated sedge). The inner ring shows the two different periods of the permafrost dynamics. The recovery period (white) is from sedge dominated period to the Betula nana dominated stage via Sphagnum dominated stage, and the black part is the Degradation period. The arrows represent the direction of succession.*

6.2.2 From Betula nana to Eriophorum angustifolium: a shift with a quick permafrost degradation

In Chapter 2, a fast vegetation shift in the *B. nana* removal plots of the nine-year *B. nana* removal experiment (2007-2015) is described, while the vegetation composition of the control plots has been stable. The dominant plant species in the removal plots shifted from *B. nana* to *A. latifolia* grass and then to *E. angustifolium* sedge (Fig. 2.5). Shrub shading in the lowland tundra reduces active layer thickness by offsetting air warming (Jorgenson et al., 2010; Myers-Smith and Hik, 2013). The *B. nana* removal treatment simulated the natural death of the deciduous shrubs and triggered the thawing of the ice-rich permafrost in the removal plots, leading to significant soil subsidence and increasing soil moisture and active layer thickness (Nauta et al., 2015). Although the increase of the active layer thickness (mid-July) halted since 2011 (Fig. 2.4), the soil subsidence continued in the removal plots (Fig. 2.4). Over 8 years, the permafrost table of the removal plots lowered on average by over 30 cm (Chapter 2). Significantly increased soil moisture caused by the dramatic permafrost thaw resulted in important vegetation composition shifts. *E. angustifolium* sedges became the dominating plant species in most of the removal plots after all *B. nana* shrubs were removed. Consequently, the vegetation composition of the removal plots became more similar to the naturally formed ponds which had developed at our site due to abrupt small-scale permafrost collapse.

6.2.3 The Arctagrostis latifolia dominated stage: a transition period driven by permafrost degradation

During the period of permafrost thawing, *A. latifolia* density increased rapidly (Chapter 2). Even though I enlist *A. latifolia* grass as one of the four symbolic plant types in the vegetation succession

cycle (Fig. 6.1), the *A. latifolia* dominated stage is just a relatively short transitional period. In the *B. nana* dominated stage, *A. latifolia* grasses usually co-exist with *B. nana* shrubs, even though the grass density is rather low. After the *B. nana* removal treatment, A. latlifolia grass cover increased to over 50% of the removal plots in 2011 (5 years after the *B. nana* removal treatment), but this number quickly dropped back to around 20% in 2015 (Fig 2.2). The short *A. latifolia* dominated period is largely attributed to the fast permafrost degradation and the relatively high nutrient release during this period. Kemper and Macdonald (2009) stated that *A. latifolia* grass is a pioneer species that quickly responds to disturbances in the Arctic tundra region. The decline of the *B. nana* cover and thicker active layer provided both open space and extra nutrients, which stimulated the growth of *A. latifolia* grasses rapidly. In another field experiment at the study site, *A. latifolia* responded strongly to a fertilization treatment, which confirms that it can respond quickly to an increased nutrient availability (Wang et al., 2016). Accordingly, the rapid increase of *A. latifolia* grass can be the signal of the initiation of the abrupt small-scale permafrost collapse in the Arctic lowland tundra. Nevertheless, the fast increase of soil moisture due to permafrost thawing might finally have exiled the *A. latifolia* grasses, which were replaced by *E. angustifolium* sedges at last.

6.2.4 Sphagnum moss: an environmental engineer on vegetation succession and permafrost recovery

In contrast to *A. latifolia*, *Sphagnum* mosses play an important role in both the vegetation succession and the permafrost recovery in the Arctic lowland tundra. In Chapter 3, a four-year *Sphagnum* addition experiment (Chapter 3) was used to imitate the invasion of *Sphagnum* mosses into the sedge dominated areas, which is common in the Arctic lowland tundra. In the experiment, *Sphagnum* mosses not only suppressed the growth of the sedges but also facilitated the germination of the woody plants like *B. nana* and *Salix* spp. shrubs (Fig. 3.4). I observed that in the plots with *Sphagnum* moss carpets, the densities of the *E. angustifolium* sedges were significantly lower than those in the plots without *Sphagnum* moss carpets (Fig. 3.3). This phenomenon can probably be explained by the reduced nutrient availability, since *Sphagnum* moss carpets are able to absorb efficiently the nutrients from water (Svensson, 1995) and atmospheric deposition (Heijmans et al., 2002).

While the growth of the sedges was suppressed by *Sphagnum* mosses, the *Sphagnum* moss carpets in the lowland tundra facilitated the germination of the *B. nana* shrubs. In the *Sphagnum* addition experiment described in Chapter 3, most of the germinated vascular plant seedlings were found in the plots with *Sphagnum* moss carpets (Fig. 3.A2). It is suggested that *Sphagnum* moss carpets provide suitable moist but unsaturated substrates that are appropriate for the germination of shrub seedlings (Oberbauer and Miller, 1982). As the wind-dispersed seeds of *B. nana* are only between 0.1 and 0.3 mg (Groot et al., 1997), it is difficult for these tiny seeds with little carbohydrate reserves to produce deep roots to reach soil water in a drying environment with extremely short growing seasons (Billings and Mooney, 1968). Therefore, the presence of the *Sphagnum* moss carpets are probably the key to the germination of the *B. nana* seeds.

In addition, the presence of *Sphagnum* mosses also results in the recovery of the degraded permafrost in the Arctic lowland tundra. Due to their high insulating capacity (van Breemen, 1995), *Sphagnum* moss carpets are able to cool the permafrost and facilitate the ice accumulation. While

permafrost thawing was not alleviated by the newly formed *Sphagnum* moss carpets in the former sedge dominated areas, the established *Sphagnum* moss carpets markedly decreased both the soil temperature (Chapter 3, Fig. 3.A2) and the active layer (Fig. 3.2). Dry *Sphagnum* moss has a strong insulating capacity, but wet *Sphagnum* moss can more easily conduct heat to deeper soil layers. This implies that the ice accumulation and permafrost recovery driven by *Sphagnum* moss carpets is expected to be a rather slow process.

6.2.5 The growth, reproduction and establishment of B. nana shrubs in the lowland tundra

In Chapter 3, it is suggested that *Sphagnum* moss facilitates the germination of *B. nana* seedlings. However, there were still two critical questions which needed to be answered. The first one was whether the sexual reproduction of *B. nana* by seeds is frequent in the Arctic lowland tundra, even though I found the young *B. nana* and other shrub seedlings at almost every plot covered by *Sphagnum* moss carpets (Chapter 3). The conventional consensus suggests that the sexual reproduction of deciduous shrubs in the Arctic tundra is rare or even not existing, mainly due to the extremely low summer temperature at these high latitudes (Billings and Mooney, 1968; Bliss, 1971; Callaghan and Collins, 1976; Bell and Bliss, 1980; Moulton and Gough, 2015), but this assumption is still controversial and rarely tested. Our study using the genetic tools (microsatellite markers) presented in Chapter 4 suggests that the sexual reproduction of *B. nana* by seeds is frequent at our site. Only 2 out of the total 210 *B. nana* ramet samples collected at the study site (near Kytalyk station) had the same genotype (Table 4.2), and these two samples were only 2 meters apart, implying that most of the samples belong to different *B. nana* individuals. Vegetative recruitment of *B. nana*, which is also very common at the study site (Li., personal observation), is probably only able to bridge very short distances. The study by Douhovnikoff et al. (2010) also found a high sexual reproduction frequency of *Salix* spp. shrubs in the Arctic tundra. Sexual reproduction of deciduous shrubs at the northern high latitudes is probably much more frequent than previously expected.

There is apparently a high gene flow among the different patches at the same site (Chapter 4). The light wind-dispersed seeds and pollens of *B. nana* can be easily transported to the sites around over distances of a few hundred meters (Molau and Larsson, 2000). I further found that long geographic distances hamper but do not prohibit gene flow. A large genetic differentiation between the samples from the Kytalyk station and those from the Kodak research station 40 km apart was found (Fig. 4.3), while the gene flow between these two sites still exists according to the result of the AMOVA tests (estimated variance among populations: 0.042, Chapter 4).

The fierce competition between woody plant seedlings and *Sphagnum* mosses at these northern ecosystems often lead to extremely high seedling mortality (van Breemen, 1995; Gunnarsson and Rydin, 1998; Ohlson et al., 2001). Therefore, the second question is whether and how *B. nana* seedlings outcompete *Sphagnum* mosses and eventually become the dominating plants. Fortunately, the dendrochronological data collected at the same site gives us some clues to understand this process (Chapter 5). The significant correlation between the mean summer (June-August) precipitation per five-year period and the total number of the *B. nana* shrubs established during the same period (Fig. 5.4) implied that *B. nana* seedlings probably have higher chances to survive during few-year long periods with relatively high summer precipitation. High summer

precipitation not only creates moist substrates for shrub germination and ramet sprouting (Chapter 3), but also stimulates the growth of *B. nana* seedlings during warm summers (Chapter 5). Based on the dendrochronological data, we also found that summer temperature, especially early summer temperature (mid-June to mid-July) is critical to the radial growth of *B. nana* (Fig. 5.3). To sum up, suitable climate conditions including relatively high summer precipitation and high temperatures are the key factors for the establishment of *B. nana* shrubs in the *Sphagnum* moss dominated areas.

6.3 Vegetation succession and greenhouse gas emission

Vegetation shifts had significant influence on the greenhouse gas fluxes especially CH_4 in the Arctic tundra. In the *Sphagnum* removal experiment described in Chapter 3, the presence of *Sphagnum* moss carpets significantly decreased the CH_4 emission (Fig. 3.4). Aerobic unsaturated *Sphagnum* carpets are perfect substrates for endophytic CH_4-oxidizing bacteria (Raghoebarsing et al., 2005; Kip et al., 2010). Another reason is that *Sphagnum* mosses also suppress the growth of *E. angustifolium* sedges (Fig. 3.3), which can facilitate the release of CH_4 through their aerenchyma which provide a direct pathway from deep soil to the atmosphere (Ström et al., 2005). In the study done by Nauta et al. (2015), the CH_4 flux had significantly increased a few years after the *B. nana* removal treatment (the same experiment described in Chapter 2). Thus, at the different stages of the vegetation succession loop in the Arctic lowland tundra (Fig. 6.1), the greenhouse gas fluxes might be distinctively different.

6.4 Recommendations

In conclusion, this study strongly suggests a complete vegetation succession cycle at a Siberian Arctic lowland tundra site through the use of several complementary approaches. This vegetation cycle appeared to be strongly connected with cycles in the local permafrost, and is affected by climate factors such as summer precipitation and temperature. In the recent years, researchers began to notice the importance of thermokarst processes such as abrupt small scale permafrost collapse (Jorgenson et al., 2006), which triggers the vegetation succession loop revealed in this thesis. However, it is questionable what exactly triggers the abrupt permafrost collapse inside the *B. nana* dominated patches. Many recent studies (Jorgenson et al., 2006; Schuur et al., 2015) stress the close relationship between increasing air temperature and frequent abrupt permafrost collapse in the Arctic tundra. At the research site (Kytalyk), the small-scale abrupt permafrost collapses are widespread. One of the plausible reasons is the degradation of *B. nana* shrub cover. The loss of protection by reduced shrub shading might initiate small-scale permafrost collapse. Other unknown biotic or abiotic factors are probably also involved. For our understanding of the impacts of climate change it is crucial to investigate the factors that trigger the abrupt permafrost collapse and affect the interaction between vegetation succession and permafrost stability in the Arctic lowland tundra.

Further studies on the rate of the vegetation succession in the Arctic lowland tundra are required. Vegetation succession is proved to have significant influences on the local greenhouse gas emission at the Arctic lowland tundra in this thesis. Recently a lot of attention has been given to thermokarst processes and associated CH_4 emission, which are increasingly incorporated in vegetation models

(van der Kolk et al., 2016). However, thermokarst features such as thaw ponds can also disappear due to vegetation succession as described in this thesis. The paleoecological studies (Teltewskoi et al., 2016) so far can only provide some evidence that depicts a rough picture of vegetation succession and environment shifts in the past few thousand years, and the different proxies used for the analyses sometimes provide contradicting information. Therefore, long-term field observations and more accurate remote-sensing analyses are required to understand the vegetation succession in Arctic lowland tundra.

Whereas the vegetation succession loop is clearly depicted in this thesis, multiple important reports and studies (Sturm et al., 2001; Walker et al., 2005; Tape et al., 2006) describe the rapid shrub expansion in the pan-Arctic region. Abrupt permafrost collapse inside the shrub patches, which subsequently leads to a rapid vegetation shift as shown in this thesis, might partly compensate the impacts of the rapid shrub expansion in the lowland tundra. Therefore, taking into account the abrupt permafrost collapse and the subsequent vegetation succession is critical for evaluating climate change effects on the whole tundra ecosystem.

References

ACIA (2005) Arctic climate impact assessment scientific report. Cambridge University Press, Cambridge

Addicott F, Lyon J (1973) Physiological ecology of abscission. In: Kozlowski TT (ed) Shedding of plant parts. Academic Press, New York and London, pp 85-124

Alsos IG, Eidesen PB, Ehrich D, Skrede I, Westergaard K, Jacobsen GH, Landvik JY (2007) Frequent long-distance plant colonization in the changing Arctic. Science 316:1606-1609

Anisimov O, Reneva S (2006) Permafrost and changing climate: the Russian perspective. Ambio 35:169-175

Anisimov OA, Lobanov VA, Reneva SA, Shiklomanov NI, Zhang T, Nelson FE (2007) Uncertainties in gridded air temperature fields and effects on predictive active layer modeling. J Geophys Res 112:261-263. doi: 10.1029/2006jf000593

Bär A, Pape R, Bräuning A, Löffler J (2008) Growth-ring variations of dwarf shrubs reflect regional climate signals in alpine environments rather than topoclimatic differences. J Biogeogr 35:625-636. doi: 10.1111/j.1365-2699.2007.01804.x

Beck I, Ludwig R, Bernier M, Lévesque E, Boike J (2015) Assessing permafrost degradation and land cover changes (1986–2009) using remote sensing data over Umiujaq, Sub-Arctic Québec. Permafrost and Periglacial Processes 26:129–141

Bell KL, Bliss L (1980) Plant reproduction in a high arctic environment. Arct Alp Res:1-10

Berner LT, Beck PSA, Bunn AG, Goetz SJ (2013) Plant response to climate change along the forest-tundra ecotone in Northeastern Siberia. Global Change Biol 19:3449-3462. doi: 10.1111/gcb.12304

Bilbrough CJ, Welker JM, Bowman WD (2000) Early spring nitrogen uptake by snow-covered plants: a comparison of Arctic and Alpine plant function under the snowpack. Arct Antarct Alp Res 32:404-411. doi: 10.2307/1552389

Billings WD (1987) Constraints to plant growth, reproduction, and establishment in Arctic environments. Arct Alp Res 19:357-365. doi: 10.2307/1551400

Billings WD, Mooney HA (1968) The ecology of Arctic and Alpine plants. Biological Reviews 43:481-529. doi: 10.1111/j.1469-185X.1968.tb00968.x

Bintanja R, Selten FM (2014) Future increases in Arctic precipitation linked to local evaporation and sea-ice retreat. Nature 509:479-482

Bliss LC (1971) Arctic and Alpine plant life cycles. Annu Rev Ecol Syst 2:405-438. doi: 10.2307/2096935

Blok D, Heijmans MMPD, Schaepman-Strub G, Kononov AV, Maximov TC, Berendse F (2010) Shrub expansion may reduce summer permafrost thaw in Siberian tundra. Global Change Biology 16:1296-1305. doi: 10.1111/j.1365-2486.2009.02110.x

Blok D, Sass-Klaassen U, Schaepman-Strub G, Heijmans MMPD, Sauren P, Berendse F (2011a) What are the main climate drivers for shrub growth in Northeastern Siberian tundra? Biogeosciences 8:1169–1179. doi: :10.5194/bg-8-1169-2011

Blok D, Schaepman-Strub G, Bartholomeus H, Heijmans MMPD, Maximov TC, Berendse F (2011b) The response of Arctic vegetation to the summer climate: relation between shrub cover, NDVI, surface albedo and temperature. Environ Res Lett 6

Blok D, Weijers S, Welker JM, Cooper EJ, Michelsen A, Löffler J, Elberling B (2015) Deepened winter snow increases stem growth and alters stem δ 13 C and δ 15 N in evergreen dwarf shrub Cassiope tetragona in high-arctic Svalbard tundra. Environ Res Lett 10

Bradley RS, Jones PD (1992) Climate since A. D. 1500. Routledge, London

Breeuwer A, Heijmans MPD, Robroek BM, Berendse F (2010) Field simulation of global change: transplanting northern bog mesocosms southward. Ecosystems 13:712-726. doi: 10.1007/s10021-010-9349-y

Bret-Harte MS et al. (2008) Plant functional types do not predict biomass responses to removal and fertilization in Alaskan tussock tundra. J Ecol 96:713-726. doi: 10.1111/j.1365-2745.2008.01378.x

Briffa KR et al. (2008) Trends in recent temperature and radial tree growth spanning 2000 years across northwest Eurasia. Philosophical Transactions: Biological Sciences 363:2271-2284. doi: 10.2307/20208637

Brubaker LB, Anderson PM, Hu FS (1995) Arctic tundra biodiversity: a temporal perspective from late Quaternary pollen records. Ecological studies : analysis and synthesis (USA) 113:111-125

Buchwal A, Rachlewicz G, Fonti P, Cherubini P, Gärtner H (2013) Temperature modulates intra-plant growth of Salix polaris from a high Arctic site (Svalbard). Polar Biol 36:1305-1318. doi: 10.1007/s00300-013-1349-x

Bunn AG (2008) A dendrochronology program library in R (dplR). Dendrochronologia 26:115-124. doi: http://dx.doi.org/10.1016/j.dendro.2008.01.002

Bunn AG, Goetz SJ (2006) Trends in satellite-observed circumpolar photosynthetic activity from 1982 to 2003: the influence of seasonality, cover type, and vegetation density. Earth Interactions 10:1723–1731

Callaghan TV, Collins NJ (1976) Strategies of growth and population dynamics of tundra plants. 1. introduction. Oikos 27:383-388. doi: 10.2307/3543457

Chapin FS et al. (2000) Arctic and boreal ecosystems of western North America as components of the climate system. Global Change Biol 6:211-223. doi: 10.1046/j.1365-2486.2000.06022.x

Chapin FS (1980) Nutrient allocation and responses to defoliation in tundra plants. Arct Alp Res 12:553-563. doi: 10.2307/1550500

Chapin FS, Schulze E-D, Mooney HA (1990) The ecology and economics of storage in plants. Annu

Rev Ecol Syst 21:423-447. doi: 10.2307/2097032

Chapin FS, Shaver GR (1996) Physiological and growth responses of Arctic plants to a field experiment simulating climatic change. Ecology 77:822-840. doi: 10.2307/2265504

Chapin FS et al. (2005) Role of land-surface changes in Arctic summer warming. Science 310:657-660. doi: 10.1126/science.1117368

Clymo RS (1970) The growth of *Sphagnum*: methods of measurement. J Ecol 58:13-49. doi: 10.2307/2258168

Cook ER, Holmes RL (1986) Users manual for program ARSTAN. Laboratory of tree-ring research. University of Arizona, Tucson

Dormann CF, Woodin SJ (2002) Climate change in the Arctic: using plant functional types in a meta-analysis of field experiments. Funct Ecol 16:4-17

Douhovnikoff V, Goldsmith GR, Tape KD, Huang C, Sur N, Bret-Harte MS (2010b) Clonal Diversity in an Expanding Community of Arctic Salix spp. and a Model for Recruitment Modes of Arctic Plants. Arct Antarct Alp Res 42:406-411. doi: 10.2307/40962396

Ellstrand NC, Roose ML (1987) Patterns of genotypic diversity in clonal plant species. Am J Bot 74:123-131

Elmendorf SC et al. (2012) Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. Ecol Lett 15:164-175. doi: 10.1111/j.1461-0248.2011.01716.x

Eugster W et al. (2000) Land–atmosphere energy exchange in Arctic tundra and boreal forest: available data and feedbacks to climate. Global Change Biol 6:84-115. doi: 10.1046/j.1365-2486.2000.06015.x

Fedorov AN, IvANOVA RN, Park H, Hiyama T, Iijima Y (2014) Recent air temperature changes in the permafrost landscapes of Northeastern Eurasia. Polar Science 8:114-128. doi: http://dx.doi.org/10.1016/j.polar.2014.02.001

Fraser RH, Lantz TC, Olthof I, Kokelj SV, Sims RA (2014) Warming-induced shrub expansion and lichen decline in the Western Canadian Arctic. Ecosystems 17:1151-1168

Frost GV, Epstein HE (2014) Tall shrub and tree expansion in Siberian tundra ecotones since the 1960s. Global Change Biol 20:1264-1277. doi: 10.1111/gcb.12406

Gärtner H, Lucchinetti S, Schweingruber FH (2014) New perspectives for wood anatomical analysis in dendrosciences: The GSL1-microtome. Dendrochronologia 32:47-51. doi: http://dx.doi.org/10.1016/j.dendro.2013.07.002

Gartner BL, Fsiii C, Shaver GR (1983) Demographic patterns of seedling establishment and growth of native graminoids in an Alaskan tundra disturbance. J Appl Ecol 20:965-980

Goetz SJ, Bunn AG, Fiske GJ, Houghton RA (2005) Satellite-observed photosynthetic trends across boreal North America associated with climate and fire disturbance. Proc Natl Acad Sci U S A

102:13521-13525. doi: 10.1073/pnas.0506179102

Groot WJd, Thomas PA, Wein RW (1997) *Betula nana* L. and *Betula glandulosa* Michx. J Ecol 85:241-264. doi: 10.2307/2960655

Gunnarsson U, Rydin H (1998) Demography and recruitment of Scots pine on raised bogs in eastern Sweden and relationships to microhabitat differentiation. Wetlands 18:133-141. doi: 10.1007/BF03161450

Hájek T, Ballance S, Limpens J, Zijlstra M, Verhoeven JA (2011) Cell-wall polysaccharides play an important role in decay resistance of *Sphagnum* and actively depressed decomposition in vitro. Biogeochemistry 103:45-57. doi: 10.1007/s10533-010-9444-3

Hallinger M, Manthey M, Wilmking M (2010) Establishing a missing link: warm summers and winter snow cover promote shrub expansion into alpine tundra in Scandinavia. New Phytol 186:890-899. doi: 10.1111/j.1469-8137.2010.03223.x

Harris C et al. (2009) Permafrost and climate in Europe: Monitoring and modelling thermal, geomorphological and geotechnical responses. Earth-Sci Rev 92:117–171

Hayati AA, Proctor MCF (1991) Limiting nutrients in acid-mire vegetation: peat and plant analyses and experiments on plant responses to added nutrients. J Ecol 79:75-95. doi: 10.2307/2260785

Heijmans MMPD, Klees H, Berendse F (2002a) Competition between *Sphagnum* magellanicum and Eriophorum angustifolium as affected by raised CO_2 and increased N deposition. Oikos 97:415-425. doi: 10.1034/j.1600-0706.2002.970311.x

Heijmans MMPD, Klees H, Visser Wd, Berendse F (2002b) Effects of increased nitrogen deposition on the distribution of 15N-labeled nitrogen between *Sphagnum* and vascular plants. Ecosystems 5:500-508. doi: 10.1007/s10021-002-0201-x

Hill GB, Henry GHR (2011) Responses of High Arctic wet sedge tundra to climate warming since 1980. Global Change Biol 17:276-287. doi: 10.1111/j.1365-2486.2010.02244.x

Hinzman L et al. (2005) Evidence and implications of recent climate change in northern Alaska and other Arctic regions. Clim Change 72:251-298. doi: 10.1007/s10584-005-5352-2

Hinzman LD, Ohata T, Polyakov IV, Suzuki R, Walsh JE (2013) JAMSTEC-IARC international collaboration enhancing understanding of the Arctic climate system. Polar Science 7:49-52. doi: http://dx.doi.org/10.1016/j.polar.2013.05.001

Hobbie SE, Chapin FS (1998) The response of tundra plant biomass, aboveground production, nitrogen, and CO_2 flux to experimental warming. Ecology 79:1526-1544. doi: 10.2307/176774

Hollesen J, Buchwal A, Rachlewicz G, Hansen BU, Hansen MO, Stecher O, Elberling B (2015) Winter warming as an important co-driver for *Betula nana* growth in western Greenland during the past century. Global Change Biol 21:2410-2423. doi: 10.1111/gcb.12913

Honnay O, Jacquemyn H (2008) A meta-analysis of the relation between mating system, growth form and genotypic diversity in clonal plant species. Evol Ecol 22:299-312

Hudson JMG, Henry GHR (2009) Increased plant biomass in a High Arctic heath community from 1981 to 2008. Ecology 90:2657-2663. doi: 10.1890/09-0102.1

Hudson JMG, Henry GHR, Cornwell WK (2011) Taller and larger: shifts in Arctic tundra leaf traits after 16 years of experimental warming. Global Change Biol 17:1013-1021. doi: 10.1111/j.1365-2486.2010.02294.x

Hugelius G et al. (2014) Improved estimates show large circumpolar stocks of permafrost carbon while quantifying substantial uncertainty ranges and identifying remaining data gaps. Biogeosci Disc 11:4771-4822

Huissteden JV, Dolman AJ (2012) Soil carbon in the Arctic and the permafrost carbon feedback. Current Opinion in Environmental Sustainability 4:545–551

IPCC (2013) Climate Change 2013: the physical science basis. Cambridge University Press, Cambridge

IPCC (2014) Climate change 2014. SYNTHESIS REPORT. Environmental Policy Collection 27:408

Iwahana G et al. Permafrost degradation after the 2002 wildfire in Kougarok, Seward Peninsula, Alaska. In: AGU Fall Meeting Abstracts, 2014a.

Iwahana G et al. (2014b) Geocryological characteristics of the upper permafrost in a tundra-forest transition of the Indigirka River Valley, Russia. Polar Science 8:96-113

Jónsdóttir IS, Callaghan TV, Headley AD (1996) Resource Dynamics within Arctic Clonal Plants. Ecological Bulletins:53-64. doi: 10.2307/20113183

Jia GJ, Epstein HE, Walker DA (2009) Vegetation greening in the canadian arctic related to decadal warming. J Environ Monit 11:2231-2238. doi: 10.1039/B911677J

Jonasson S (1982) Organic matter and phytomass on three north Swedish tundra sites, and some connections with adjacent tundra areas. Ecography 5:367-375. doi: 10.1111/j.1600-0587.1982.tb01050.x

Jorgenson MT, Grosse G (2016) Remote Sensing of Landscape Change in Permafrost Regions. Permafrost and Periglacial Processes 27: 324-338

Jorgenson MT et al. (2013) Reorganization of vegetation, hydrology and soil carbon after permafrost degradation across heterogeneous boreal landscapes. Environ Res Lett 8:035017

Jorgenson MT, Marcot BG, Swanson DK, Jorgenson JC, Degange AR (2015) Projected changes in diverse ecosystems from climate warming and biophysical drivers in northwest Alaska. Clim Change 130:1-14

Jorgenson MT, Racine CH, Walters JC, Osterkamp TE (2001) Permafrost degradation and ecological changes associated with a warming climate in Central Alaska. Clim Change 48:551-579. doi: 10.1023/a:1005667424292

Jorgenson MT et al. (2010) Resilience and vulnerability of permafrost to climate change. Canadian Journal of Forest Research 40:1219-1236. doi: 10.1139/X10-060

Jorgenson MT, Shur YL, Pullman ER (2006) Abrupt increase in permafrost degradation in Arctic Alaska. Geophys Res Lett 33:356-360

Juszak I, Erb AM, Maximov TC, Schaepman-Strub G (2014) Arctic shrub effects on NDVI, summer albedo and soil shading. Remote Sens Environ 153:79-89. doi: http://dx.doi.org/10.1016/j.rse.2014.07.021

Juszak I, Eugster W, Heijmans MMPD, Schaepmanstrub G (2016) Contrasting radiation and soil heat fluxes in Arctic shrub and wet sedge tundra. Biogeosci Disc 13:4049-4064

Kanevskiy M, Shur Y, Krzewinski T, Dillon M (2013) Structure and properties of ice-rich permafrost near Anchorage, Alaska. Cold Regions Science and Technology 93:1–11

Kattsov VM, Walsh JE, Chapman WL, Govorkova VA, Pavlova TV, Zhang X (2009) Simulation and projection of arctic freshwater budget components by the IPCC AR4 Global Climate Models. Journal of Hydrometeorology 8:571

Kemper JT, Macdonald SE (2009) Directional change in upland tundra plant communities 20-30 years after seismic exploration in the Canadian low-Arctic. J Veg Sci 20:557–567

Keyser AR, Kimball JS, Nemani RR, Running SW (2000) Simulating the effects of climate change on the carbon balance of North American high-latitude forests. Global Change Biol 6:185–195

Kip N et al. (2010) Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems. Nature Geosci 3:617-621. doi: http://www.nature.com/ngeo/journal/v3/n9/suppinfo/ngeo939_S1.html

Kolishchuk V (1990) Dendroclimatological study of prostrate woody plants. In: Cook E, Kairiukstis L (eds) Methods of dendrochronology applications in the environmental sciences. Kluwer, London, pp 51-55

Koven CD et al. (2011) Permafrost carbon-climate feedbacks accelerate global warming. Proceedings of the National Academy of Sciences 108:14769-14774

Kulju KKM, Pekkinen M, Varvio S (2004) Twenty-three microsatellite primer pairs for Betula pendula (Betulaceae). Mol Ecol Notes 4:471-473

Lang SI, Cornelissen JHC, Hölzer A, Ter Braak CJF, Ahrens M, Callaghan TV, Aerts R (2009) Determinants of cryptogam composition and diversity in *Sphagnum*-dominated peatlands: the importance of temporal, spatial and functional scales. J Ecol 97:299-310. doi: 10.1111/j.1365-2745.2008.01472.x

Lemke P et al. (2007) Observations: Changes in Snow, Ice and Frozen Ground.

Li B, Heijmans MMPD, Berendse F, Blok D, Maximov T, Sass-Klaassen U (2016) The role of summer precipitation and summer temperature in establishment and growth of dwarf shrub *Betula nana* in northeast Siberian tundra. Polar Biol 39: 1245-1255

Liang M et al. (2014) Importance of soil moisture and N availability to larch growth and distribution in the Arctic taiga-tundra boundary ecosystem, Northeastern Siberia. Polar Science 8:327-341. doi:

http://dx.doi.org/10.1016/j.polar.2014.07.008

Liljedahl AK et al. (2016) Pan-Arctic ice-wedge degradation in warming permafrost and its influence on tundra hydrology. Nature Geoscience 9

Limpens J, Berendse F, Klees H (2003) N deposition affects N availability in interstitial water, growth of *Sphagnum* and invasion of vascular plants in bog vegetation. New Phytol 157:339-347. doi: 10.1046/j.1469-8137.2003.00667.x

Limpens J, Berendse F, Klees H (2004) How phosphorus availability affects the impact of nitrogen deposition on *Sphagnum* and vascular plants in bogs. Ecosystems 7:793-804. doi: 10.1007/s10021-004-0274-9

Lloyd AH, Yoshikawa K, Fastie CL, Hinzman L, Fraver M (2003) Effects of permafrost degradation on woody vegetation at arctic treeline on the Seward Peninsula, Alaska. Permafrost Periglac Process 14:93-101. doi: 10.1002/ppp.446

Loranty M, Goetz S, Rastetter E, Rocha A, Shaver G, Humphreys E, Lafleur P (2011) Scaling an instantaneous model of tundra NEE to the Arctic landscape. Ecosystems 14:76-93. doi: 10.1007/s10021-010-9396-4

Mäkinen H et al. (2003) Large-scale climatic variability and radial increment variation of Picea abies (L.) Karst. in central and northern Europe. Trees 17:173-184. doi: 10.1007/s00468-002-0220-4

Mack MC, Schuur EAG, Bret-Harte MS, Shaver GR, Chapin FS (2004) Ecosystem carbon storage in Arctic tundra reduced by long-term nutrient fertilization. Nature 431:440-443

Malmer N, Svensson B, Wallén B (1994) Interactions between *Sphagnum* mosses and field layer vascular plants in the development of peat-forming systems. Folia Geobot Phytotaxon 29:483-496. doi: 10.1007/BF02883146

Marschner P, Rengel Z, Stark S (2007) Nutrient cycling in the tundra. In: Marschner P, Rengel Z (eds) Nutrient Cycling in Terrestrial Ecosystems. Springer, Berlin, pp 309-331

McGuire AD, Chapin FS, Walsh JE, Wirth C (2006) Integrated regional changes in Arctic climate feedbacks: implications for the global climate system. Ann Rev Environ Resour 31:61-91. doi: doi:10.1146/annurev.energy.31.020105.100253

Mcguire AD, Christensen TR, Hayes D, Heroult A (2012) An assessment of the carbon balance of arctic tundra: comparisons among observations, process models, and atmospheric inversions. Biogeosciences 9:3185-3204

Meinardus C, Weinert B, Löffler J, Lundberg A, Bräuning A (2011) The potential of the dwarf shrub *Betula nana* L. as a climate indicator above the tree line in the southern Norwegian Scandes. Potsdam

Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evolution 60:2399–2402

Miller H (1975) Anatomical characteristics of some woody plants of the Angmagssalik district of

Southeast Greenland. Medd Gronland 198:1-30

Miller PC, Mangan R, Kummerow J (1982) Vertical distribution of organic matter in eight vegetation types near Eagle Summit, Alaska. Ecography 5:117-124. doi: 10.1111/j.1600-0587.1982.tb01025.x

Molau U, Larsson EL (2000) Seed rain and seed bank along an alpine altitudinal gradient in Swedish Lapland. Canadian Journal of Botany 78:728-747

Moulton CA, Gough L (2015) Effects of soil nutrient availability on the role of sexual reproduction in an Alaskan tundra plant community. Arctic Antarctic and Alpine Research 43:612-620

Myers-Smith IH et al. (2015a) Climate sensitivity of shrub growth across the tundra biome. Nature Climate Change 5:887-891

Myers-Smith IH et al. (2011) Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. Environ Res Lett 6. doi: 10.1088/1748-9326/6/4/045509

Myers-Smith IH et al. (2015b) Methods for measuring Arctic and Alpine shrub growth: A review. Earth-Sci Rev 140:1–13

Myers-Smith IH, Hik DS (2013) Shrub canopies influence soil temperatures but not nutrient dynamics: An experimental test of tundra snow–shrub interactions. Ecology and Evolution 3:3683-3700

Myneni RB, Keeling CD, Tucker CJ, Asrar G, Nemani RR (1997) Increased plant growth in the northern high latitudes from 1981 to 1991. Nature 386:698-702

Nauta AL et al. (2015) Permafrost collapse after shrub removal shifts tundra ecosystem to a methane source. Nature Clim Change 5:67-70. doi: 10.1038/nclimate2446

Nelson FE, Anisimov OA, Shiklomanov NI (2001) Subsidence risk from thawing permafrost. Nature 410:889-890

Nowinski N, Taneva L, Trumbore S, Welker J (2010) Decomposition of old organic matter as a result of deeper active layers in a snow depth manipulation experiment. Oecologia 163:785-792. doi: 10.1007/s00442-009-1556-x

Oberbauer S, Miller PC (1982) Effect of water potential on seed germination. Ecography 5:218-220. doi: 10.1111/j.1600-0587.1982.tb01040.x

Ohlson M (1999) Differentiation in adaptive traits between neighbouring bog and mineral soil populations of Scots pine Pinus sylvestris. Ecography 22:178-182. doi: 10.1111/j.1600-0587.1999.tb00466.x

Ohlson M, Økland RH, Nordbakken J-F, Dahlberg B (2001) Fatal interactions between Scots pine and *Sphagnum* mosses in bog ecosystems. Oikos 94:425-432. doi: 10.2307/3547390

Olefeldt D et al. (2016) Circumpolar distribution and carbon storage of thermokarst landscapes. Nature Communications 7:13043

Oosterhout CV, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker : software for

identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Osterkamp TE (2005) The recent warming of permafrost in Alaska. Global and Planetary Change 49:187-202

Osterkamp TE, Jorgenson MT, Schuur EAG, Shur YL, Kanevskiy MZ, Vogel JG, Tumskoy VE (2009) Physical and ecological changes associated with warming permafrost and thermokarst in Interior Alaska. Permafrost and Periglacial Processes 20:235-256

Osterkamp TE, Romanovsky VE (1999) Evidence for warming and thawing of discontinuous permafrost in Alaska. Permafrost and Periglacial Processes 10:17-37

Oulehle F, Rowe EC, Myška O, Chuman T, Evans CD (2016) Plant functional type affects nitrogen use efficiency in high-Arctic tundra. Soil Biol Biochem 94:19-28. doi: http://dx.doi.org/10.1016/j.soilbio.2015.11.008

Parmentier F, Huissteden Jv, Kip N, Camp H, Jetten M, Maximov T, Dolman A (2011a) The role of endophytic methane-oxidizing bacteria in submerged *Sphagnum* in determining methane emissions of Northeastern Siberian tundra. Biogeosciences 8:1267-1278

Parmentier FJW, Huissteden JV, Kip N, Camp HJMOD (2011b) The role of endophytic methane oxidizing bacteria in submerged *Sphagnum* in determining methane emissions of Northeastern Siberian tundra. Biogeosciences 8:1267-1278

Parmentier FJW et al. (2011c) Longer growing seasons do not increase net carbon uptake in Northeastern Siberian tundra. Biogeosciences 116:1091-1095. doi: 10.1029/2011JG001653

Pearson RG, Phillips SJ, Loranty MM, Beck PSA, Damoulas T, Knight SJ, Goetz SJ (2013) Shifts in Arctic vegetation and associated feedbacks under climate change. Nature Climate Change 3:673-677

Peichl M, Sonnentag O, Nilsson M (2015) Bringing color into the picture: using digital repeat photography to investigate phenology controls of the carbon dioxide exchange in a boreal mire. Ecosystems 18:115-131. doi: 10.1007/s10021-014-9815-z

Ping CL, Michaelson GJ, Fortier D (2013) Ground ice in the upper permafrost of the Beaufort Sea coast of Alaska. Cold Regions Science and Technology 85:56-70

Pop EW, Oberbauer SF, Starr G (2000) Predicting vegetative bud break in two Arctic deciduous shrub species, *Salix pulchra* and *Betula nana*. Oecologia 124:176-184. doi: 10.1007/s004420050005

Pouliot R, Rochefort L, Karofeld E, Mercier C (2011) Initiation of *Sphagnum* moss hummocks in bogs and the presence of vascular plants: Is there a link? Acta Oecol 37:346-354. doi: http://dx.doi.org/10.1016/j.actao.2011.04.001

Qian H, Joseph R, Zeng N (2010) Enhanced terrestrial carbon uptake in the Northern High Latitudes in the 21st century from the Coupled Carbon Cycle Climate Model Intercomparison Project model projections. Global Change Biol 16:641–656

Raghoebarsing AA et al. (2005) Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. Nature 436:1153-1156

Raynolds MK et al. (2014) Cumulative geoecological effects of 62years of infrastructure and climate change in ice-rich permafrost landscapes, Prudhoe Bay Oilfield, Alaska. Global Change Biol 20:1211-1224

Robinson et al. (1995) Growth responses of *Calamagrostis lapponica* to simulated environmental change in the sub-Arctic. Oikos 72:61-66

Schuur EAG et al. (2015) Climate change and the permafrost carbon feedback. Nature 520:171-179. doi: 10.1038/nature14338

Schweingruber FH (1996) Tree rings and environment dendroecology. Paul Haupt Publishers, Berne

Schweingruber FH, Borner A, Schulze ED (2011) Altas of stem anatomy in herbs, shrubs and trees. Springer, Berlin

Schweingruber FH, Hellmann L, Tegel W, Braun S, Nievergelt D, Büntgen U (2013) Evaluating the wood anatomical and dendroecological potential of arctic dwarf shrub communities. Iawa Journal 34:485-497

Serreze MC et al. (2000) Observational evidence of recent change in the northern high-latitude environment. Clim Change 46:159-207. doi: 10.1023/A:1005504031923

Shaver GR, Bret-Harte MS, Jones MH, Johnstone J, Gough L, Laundre J, Chapin FS (2001) Species composition interacts with fertilizer to control long-term change in tundra productivity. Ecology 82:3163-3181

Shaver GR, Chapin FS (1991) Production: biomass relationships and element cycling in contrasting Arctic vegetation types. Ecol Monogr 61:1-31. doi: 10.2307/1942997

Shaver GR, Chapin FS (1986) Effect of fertilizer on production and biomass of tussock tundra, Alaska, U.S.A. Arctic and Alpine Research 18:261-268

Shaver GR, Cutler JC (1979) The vertical distribution of live vascular phytomass in cottongrass tussock tundra. Arct Alp Res 11:335-342. doi: 10.2307/1550421

Shaver GR et al. (1998) Biomass and CO_2 flux in wet sedge tundras: responses to nutrients, temperature, and light. Ecol Monogr 68:75-97

Shur YL, Jorgenson MT (2007) Patterns of permafrost formation and degradation in relation to climate and ecosystems. Permafrost and Periglacial Processes 18:7-19

Siewert MB, Hanisch J, Weiss N, Kuhry P, Maximov TC, Hugelius G (2015) Comparing carbon storage of Siberian tundra and taiga permafrost ecosystems at very high spatial resolution. Journal of Geophysical Research Biogeosciences 120:1973-1994

Sonesson M, Persson S, Basilier K, Stenström TA (1980) Growth of *Sphagnum* riparium Ångstr. in relation to some environmental factors in the Stordalen mire. Ecological Bulletins:191-207. doi: 10.2307/20112773

Steltzer H, Hufbauer RA, Welker JM, Casalis M, Sullivan PF, Chimner R (2008) Frequent sexual reproduction and high intraspecific variation in *Salix arctica*: Implications for a terrestrial feedback to climate change in the High Arctic. Journal of Geophysical Research Biogeosciences 113:1469-1495

Stow DA et al. (2004) Remote sensing of vegetation and land-cover change in Arctic tundra ecosystems. Remote Sens Environ 89:281-308. doi: http://dx.doi.org/10.1016/j.rse.2003.10.018

Ström L, Mastepanov M, Christensen TR (2005) Species-specific effects of vascular plants on carbon turnover and methane emissions from wetlands. Biogeochemistry 75:65-82

Sturm M, Douglas T, Racine C, Liston GE (2005) Changing snow and shrub conditions affect albedo with global implications. Biogeosciences 110:195-221. doi: 10.1029/2005JG000013

Sturm M, Holmgren J, McFadden JP, Liston GE, Chapin FS, Racine CH (2001) Snow–shrub interactions in Arctic tundra: a hypothesis with climatic implications. J Clim 14:336-344. doi: 10.1175/1520-0442(2001)014

Svensson BM (1995) Competition between *Sphagnum* fuscum and Drosera rotundifolia: a case of ecosystem engineering. Oikos 74:205-212. doi: 10.2307/3545649

Tape KEN, Sturm M, Racine C (2006) The evidence for shrub expansion in Northern Alaska and the Pan-Arctic. Global Change Biol 12:686-702. doi: 10.1111/j.1365-2486.2006.01128.x

Tarnocai C, Canadell JG, Schuur EAG, Kuhry P, Mazhitova G, Zimov S (2009) Soil organic carbon pools in the northern circumpolar permafrost region. Global Biogeochemical Cycles 23:2607-2617

Teltewskoi A et al. (2016) 4000 Years of Changing Wetness in a Permafrost Polygon Peatland (Kytalyk, NE Siberia): A comparative high‐resolution multi‐proxy study. Permafrost and Periglacial Processes 27:76-95

Treat CC, Bubier JL, Varner RK, Crill PM (2007) Timescale dependence of environmental and plant-mediated controls on CH4 flux in a temperate fen. Journal of Geophysical Research: Biogeosciences 112:G01014. doi: 10.1029/2006JG000210

Truong C, Palmé AE, Felber F, Naciri-Graven Y (2005) Isolation and characterization of microsatellite markers in the tetraploid birch, Betula pubescens ssp. tortuosa. Mol Ecol Notes 5:96-98

Urban M, Forkel M, Eberle J, Hüttich C, Schmullius C, Herold M (2014) Pan-Arctic climate and land cover trends derived from multi-variate and multi-scale analyses (1981–2012). Remote Sensing 6:2296

van Breemen N (1995) How *Sphagnum* bogs down other plants. Trends Ecol Evol 10:270-275. doi: http://dx.doi.org/10.1016/0169-5347(95)90007-1

van der Kolk H-J, Heijmans MMPD, van Huissteden J, Pullens JWM, Berendse F (2016) Potential Arctic tundra vegetation shifts in response to changing temperature, precipitation and permafrost thaw. Biogeosciences 13:6229-6245. doi: 10.5194/bg-13-6229-2016

van Huissteden J, Maximov TC, Dolman AJ (2005) High methane flux from an arctic floodplain

(Indigirka lowlands, eastern Siberia). Journal of Geophysical Research Biogeosciences 110:565-571

van Wijk MT et al. (2004) Long-term ecosystem level experiments at Toolik Lake, Alaska, and at Abisko, Northern Sweden: generalizations and differences in ecosystem and plant type responses to global change. Global Change Biol 10:105-123

Wahren CHA, Walker MD, Bret-Harte MS (2005) Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. Global Change Biology 11:537-552. doi: 10.1111/j.1365-2486.2005.00927.x

Walker DA (2000) Hierarchical subdivision of Arctic tundra based on vegetation response to climate, parent material and topography. Global Change Biol 6:19-34

Walker DA, Billings WD, De Molenaar JG (1997) Snow–vegetation interactions in tundra environments. In: Jones HG, Pomeroy JW, Walker DA, Hoham RW (eds) Snow ecology: an interdisciplinary examination of snow-covered ecosystems. Cambridge University Press, New York, pp 266-324

Walker DA et al. (2005) The circumpolar Arctic vegetation map. J Veg Sci 16:267-282. doi: 10.1111/j.1654-1103.2005.tb02365.x

Walker MD et al. (2006) Plant community responses to experimental warming across the tundra biome. Proc Nat Acad Sci Usa 103:1342-1346. doi: 10.1073/pnas.0503198103

Wang P et al. (2016a) Above- and below-ground responses of four tundra plant functional types to deep soil heating and surface soil fertilization. J Ecol:n/a-n/a. doi: 10.1111/1365-2745.12718

Wang P, Mommer L, Ruijven Jv, Berendse F, Maximov TC, Heijmans MMPD (2016b) Seasonal changes and vertical distribution of root standing biomass of graminoids and shrubs at a Siberian tundra site. Plant Soil:1-11

Williams M, Eugster W, Rastetter EB, Mcfadden JP, Chapin FSC (2000) The controls on net ecosystem productivity along an Arctic transect: a model comparison with flux measurements. Global Change Biol 6:116-126

Woodcock H, Bradley RS (1994) Salix arctica (Pall): Its potential for dendroclimatological studies in the High Arctic. Dendrochronologia 12:11-22

Yoshikawa K, Hinzman LD (2003) Shrinking thermokarst ponds and groundwater dynamics in discontinuous permafrost near council, Alaska. Permafrost and Periglacial Processes 14:151–160

Zackrisson O, Nilsson M-C, Dahlberg A, Jäderlund A (1997) Interference mechanisms in conifer-Ericaceae-Feathermoss Communities. Oikos 78:209-220. doi: 10.2307/3546287

Zhang T, Barry RG, Knowles K, Heginbottom JA, Brown J Taylor and Francis Online : Statistics and characteristics of permafrost and ground‐ice distribution in the Northern Hemisphere-Polar Geography-Volume 23, Issue 2. Polar Geography

Summary

Arctic ecosystems have been affected by severe climate change during the last decades. The increase in temperature in the Arctic has been almost double of the global rate of warming since the beginning of the 20th century. Like other ecosystems in the high latitude region, Arctic tundra appears to be extremely sensitive to the continuous warming of the past decades, which has led to dramatic vegetation changes such as rapid shrub expansion. While researchers are keen to talk about the shrubification of the Arctic tundra, there has been rather little attention for alternative vegetation shifts, such as those related to local permafrost collapse in lowland tundra. The general vegetation succession route of the ice-rich lowland tundra ecosystem is yet largely unknown. Therefore, we choose a typical Arctic lowland site (Kytalyk natural reserve) in the Northeastern Siberia to explore how vegetation is changing in this ecosystem, and how changes in the abiotic environment and vegetation succession interact.

On the basis of field observations I assumed that the plant species composition of each vegetation patch at the study site changes continuously following cycles over time. To test this assumption, two multiple-year field experiments (Chapter 2 and Chapter 3) were carried out. In addition, we applied dendrochronological techniques (Chapter 4 and Chapter 5) and molecular tools (Chapter 4). On the basis of the results of these studies, I depicted a complete vegetation succession loop in the Arctic lowland tundra, which is closely related to the dynamics of the permafrost. In this vegetation succession loop, four stages with distinctive vegetation types have been identified.

The *Betula nana* L. shrubs mainly dominate the well-drained elevated areas. In a field experiment, removal of *B. nana* shrubs resulted in abrupt permafrost degradation, rapid soil moisture increase and invasion of the grass species *Arctagrostis latifolia* (R. Br.) Griseb. After a short time period, when small ponds or drainages had developed, this fast-responding grass species is replaced by *Eriophorum* sedges. In the subsequent stage the *Sphagnum* mosses invade the sedge vegetation. The new *Sphagnum* moss carpets not only suppress the growth of *Eriophorum* sedges, but also create moist but unsaturated substrates that appear to be appropriate for the germination of *B. nana* seeds. These conditions provide new opportunities for *B. nana* shrubs to establish.

The reproduction mode of *B. nana* at the study site has been studied using molecular tools (microsatellites), as it may explain how existing *B. nana* patches developed and how shrub vegetation may expand in the future (Chapter 4). The conventional point of view is that sexual reproduction of perennial plants in the Arctic tundra, like *B. nana,* is rare due to the pressure of the harsh environment. However, the results of our molecular study (Chapter 4) tell a different story. While vegetative reproduction of *B. nana* is common, sexual reproduction of *B. nana* is more prevalent. Seed dispersal of *B. nana* between different patches at the study site is not hampered by the short between-patch distances, but vegetative reproduction of *B. nana* appeared to be restricted to 1-2 m distances from the parent plants.

The influences of the climate on *B. nana* shrubs were further investigated using the dendrochronological analyses (Chapter 4 and Chapter 5). The radial growth of *B. nana* is positively correlated with early summer temperature, while relatively high summer precipitation during the warm years also stimulates the growth of *B. nana*. Moreover, sufficient summer precipitation

facilitates the establishment of *B. nana* seedlings. Since sexual reproduction is prevalent at the site, it is suggested that the present *B. nana* shrubs established simultaneously, during periods with suitable climate conditions.

Along with the vegetation succession cycles, permafrost underlying the vegetation experiences clear degradation-recovery cycles. We detected a close interaction between vegetation shifts and permafrost dynamics. While abrupt permafrost degradation drove a quick vegetation shift from the *B. nana* dominated stage to the water-logged *Eriophorum* sedge dominate stage, the changes of vegetation cover affect the stability of the permafrost as well. The removal of *B. nana* shrub cover triggered rapid permafrost degradation (Chapter 2), while the development of *Sphagnum* moss carpets, which have a high isolation capacity, reduced permafrost temperature, facilitating permafrost recovery (Chapter 3).

Vegetation composition in the Arctic tundra not only influences permafrost stability, but also affects the methane emission of the site. *Eriophorum* sedges are able to transport methane from deep soil to the air via their aerenchyma tissues, leading to high methane fluxes. In contrast, the *Sphagnum* mosses significantly suppress the methane emission, since endophytic CH_4-oxidizing bacteria are widespread inside the aerobic unsaturated *Sphagnum* carpets (Chapter 3).

To sum up, our findings provide crucial information to better understand changes in the Arctic tundra ecosystem, helping to obtain better predictions of future vegetation shifts and the associated consequences for greenhouse gas emissions, permafrost stability and the heat balance of the Earth surface.

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At the beginning of 2010, I had just finished my fieldwork for my master thesis in South Africa. It was an amazing but tough journey. I swore to myself that I had to find some other places without millions of insects and extremely hot weather. It is probably the initial reason why I chose to do research in Siberia (Well, I was chosen by Monique and Frank luckily.). Nevertheless, I was completely wrong. In Kytalyk, our tundra research site in the Northeastern Siberia, we have to bear trillions of mosquitos, instead of millions of them, every single day. The weather in summer is like Forrest Gump's chocolate box, totally unpredictable. The temperature can reach 30 °C today but drop back to freezing tomorrow.

However, I have never regretted to be there. The ecosystem in Siberia is breathtaking and full of surprise. Although I have not had chances to see aurora yet, the orange mid night sunshine is also incredibly beautiful. Doing research in Siberia is so far the biggest adventure in my life. Therefore, I want to thank my promoter Prof. Frank Berendse and daily Supervisor dr. Monique Heijmans for choosing me to work in such a remote, harsh but also extremely wild and beautiful place, but also for all support that they provided.

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SENSE PhD Courses

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  on eastern Eurasia"' (2014)

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o Introduction to R for statistical analysis, Wageningen University (2012)
o Soil Ecology, Wageningen University (2012)
o Wood Anatomy in Arctic and Alpine Environments, Swiss Federal Institute for Forest,
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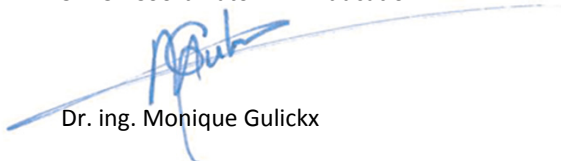o Competence Assessment, Wageningen University (2011)

Management and Didactic Skills Training

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  temperature on soil temperature in the Siberian tundra landscape?' (2013)

Oral Presentations

o *Clash of Plants: the role of Sphagnum in the vegetation dynamics and greenhouse gas
  emission in Northeast Siberia.* 8th Annual International Symposium on C/H2O/Energy
  balance and climate over boreal and arctic regions with special emphasis on eastern
  Eurasia, 10-12 November 2014, Wageningen, The Netherlands
o *Climatic factors influencing radial growth of Betula nana in tundra ecosystem.* Tree Rings
  in Archaeology, Climatology and Ecology (TRACE 2014), 6-10 May 2014, Aviemore, UK
o *A story of the permafrost small-scale collapse at the deciduous shrub patches in the
  northeast Siberian tundra.* European Geosciences Union (EGU) General Assembly 2014,
  27 April - 2 May 2014, Vienna, Austria

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