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Snow Depth and Vegetation Cover Effects on Ecological Functioning in Headwater Streams

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Biology	
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Abstract

I studied the possible effects of reduced snow depth on ecological functioning including ecological processes like algal growth and the rates of grazing and decomposition in boreal headwater streams in the Dovre Mountains in Central Norway. The streams drained catchments of three vegetation types, forest, shrub and tundra. Measurements of ecological processes (leaf-litter decomposition, algal growth and grazing rates) and analysis of stable isotopes (SIA) were performed in manipulated streams and in unmanipulated control streams. Leaf-litter decomposition by leaf-eating macroinvertebrates and microbial agents was assessed using enclosed mesh bags. Algal growth and grazing rates by macroinvertebrates were assessed by measuring algal growth (as the accrued amount of Chlorophyll a) on pairs of unglazed ceramic tiles placed onto the stream bed. The trophic importance of riparian vs. autochthonous food sources that support macro-invertebrate primary consumers was analyzed using dual stable isotope signatures, i.e. I measured stable carbon (δ^{13} C) and nitrogen (δ^{13} N) isotope profiles for three carbon and nutrient sources and five primary consumers.

The results showed that specialized macroinvertebrates contributed in a much greater extent than microbial agents to rates of decomposition by leaf litter in all vegetation covers. Further, that the amount of shading and snow depth manipulation may affect decomposition rates in streams draining forest and tundra positively and negatively, respectively.

A drought-period the summer after snow depth manipulation caused most of the studied streams in various degrees to dry in. Hence, the number of measurements was not sufficient to test whether primary production or grazing rates varied with vegetation cover or by manipulation of snow depth. All macro-invertebrates species differed in their isotopic profile. However, I was unable to test whether these differences was due to different diets due to the limited variation of isotopic profiles of potential food sources. The results of this study did not show that a reduction of snow cover would cause alterations of ecological functioning in streams draining different vegetation covers. However, both vegetation cover (shading) alone and the interaction of vegetation cover and manipulation were significant for decomposition of leaf-litter. Further, it supports the important role vegetation cover is expected to have on important processes by affecting the micro climate along the stream and through input of organic materials. Hence, for us to understand and, if possible, predict how stream ecosystems responds to local and regional changes of climate and land use, there is still a need of more knowledge about these processes.

Sammendrag

Vintre med stabilt snødekke er et karakteriserende kjennetegn for områder i overgangen mellom skog og tundra i alpine områder. Men med de pågående klimaendringene er det forventet at den snødekte perioden endres ved at timingen forandres, og lengden og tykkelsen reduseres. Videre er ulike vegetasjonstyper vist å påvirke økologisk funksjon i bekker, blant annet ved å gi skygge og tilføre organisk materiale. I denne studien tok jeg for meg mulige effekter av endret snødekke på økologisk funksjon i bekker, som primærproduksjon (algevekst) og betydningen av beiting og nedbrytning, i alpine bekker i tre ulike vegetasjonstyper; skog, vierkratt og tundra. For å komplementere dette undersøkte jeg profiler av karbon- og nitrogen-isotoper i potensielle næringskilder og utvalgte bentiske makro-invertebrater. Resultatene viste at spesialiserte makro-invertebraters bidrag til nedbrytning av utsatt løvmateriale var betydelig større enn mikrobers. Videre ble det vist at mengden skygge og en reduksjon av snødekket kan påvirke nedbrytningen positivt og negativt i respektivt skog og tundra. Selv om vegetasjonstype virker å være den viktigste faktoren, kan det ikke utelukkes at snødybde-manipulasjon kan ha hatt en effekt på nedbrytningen. Ingen endring i nedbrytning ble funnet i vierkratt. En tørkeperiode i løpet av sommeren etter snødybde-manipuleringen førte til at mange av bekkene som inngikk i studiet tørket delvis eller helt inn. Dette førte til at det ikke var mulig å undersøke om primærproduksjon, eller beiting av algemateriale og biofilm varierte med vegetasjonstype eller snødybde-manipulasjon. Stabil isotop-analysen viste at alle de innsamlede makroinvertebratene hadde hver sin isotopprofil. Det var derimot ikke mulig å teste om forskjellen skyltes ulik diett da de innsamlede potensielle næringskildene, løvmateriale av bjørk (*Betula* spp.) og vier (*Salix* spp.), innsamlet i og ved siden av bekken, generelt ikke varierte i sammensetningen av nitrogen- og karbon- isotoper mellom prøver av samme slekt eller mellom slektene. Resultatene viste ikke en entydig effekt av endret snødekke på økologiske prosesser i bekker som renner gjennom ulike vegetasjonstyper. Men de viste at vegetasjonstype for seg selv, og sammen med snødybde, kan ha påvirket blant annet nedbrytning av løvmateriale. Dette funnet følger antagelsene om at vegetasjonstype spiller en betydelig rolle i flere viktige økologiske prosesser gjennom å påvirke mikroklimaet i og rundt bekkene og bidrag til tilførsel av organisk materiale. Det vil derfor være et fortsatt behov for mer kunnskap om disse prosessene for å kunne forstå og om mulig forutsi hvordan slike systemer responderer på lokale og mer globale endringer i klima og arealbruk.

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1. Introduction

Climate change affects the distribution of vegetation cover. The changes may be most easily visible at the forest-tundra transition in alpine habitats. Vegetation cover and structure is known to affect ecological functioning suggesting that climate change should not only directly, for instance via a different temperature regime, but also indirectly affect ecological functioning. I studied the combined effects of vegetation cover and reduction in snow cover on ecological functioning in headwater streams.

Climate change is expected to generally increase the amount of precipitation in Norway and may result in an increase in the proportion of rain rather than snow with concomitant effects on the number and strength of soil frost events (Haei *et al.*, 2010; IPCC, 2007). Characteristic features of the winter season in the northern hemisphere include a stable snow cover affecting abiotic and biotic processes. One of the predicted regional impacts of climate change is the alteration of the timing, extent and duration of snow cover in northern areas (Christensen *et al.*, 2007). A thinner and less extensive snow cover and the resulting reduction in soil temperature have been shown to disrupt soil microbial activity (e.g.Bölter *et al.* (2005); Clein & Schimel (1995), increase leaching of carbon (C), nitrogen (N) and phosphorus (P) (Matzner & Borken, 2008) and affect understory plant cover and community composition (Kreyling, Haei & Laudon, 2012). The alteration of precipitation patterns and the resulting consequences for the distribution and strength of soil frost may thus affect ecosystems on multiple levels.

Several studies have documented rapid land use change, for instance afforestation following a reduction in grazing pressure by large herbivores including sheep and reindeer (e.g. Gehrig-Fasel, Guisan & Zimmermann (2007); Speed *et al.* (2011)). In addition to land use change, predicted increases in temperature and precipitation (IPCC, 2007; Starfield & Chapin, 1996) suggest expansion of forest and shrub habitats towards higher altitudes in mountainous areas presently inhabited by tundra species. Forest and shrub habitats tend to accumulate more snow than tundra habitats, which are more exposed to wind and less shaded. Under stable climate conditions, the accumulation of snow among tall vegetation may ensure a stable snow cover in forest and shrub habitats, minimizing soil frost, providing protection from low soil temperatures and causing a prolonged snow cover period and late snowmelt (Kreyling, Haei & Laudon, 2012; Liston *et al.*, 2002). If precipitation shifts towards a lower proportion of snow, shrub and forest habitats may still experience snow accumulation, whereas tundra habitats may become more exposed and hence experience a more varied temperature regime.

Assessment of the ecological state of an ecosystem, i.e. of ecosystem integrity (ecological and biological integrity) may facilitate observation and evaluation of predicted changes in a given ecosystem; the state of a stream may accordingly be graded from pristine to strongly impacted (Karr, 1991). According to Minshall (1996), ecosystem integrity can be divided into structural and functional components. Whereas structural integrity relates to the qualitative and quantitative species and functional composition of biological communities, functional integrity is described by complementary process rates and patterns and the relative importance of different ecosystem-level processes under reference conditions (Gessner & Chauvet, 2002). The two components of ecosystem integrity, structural and functional, jointly describe the ecosystem as a whole. Several examples (as presented in Gessner and Chauvet (2002)) illustrate that depending on the context data on functional aspects may be more informative than data on structural patterns. For example, a study by Rodgers et al. (1979) found that primary production (function) of periphyton in outdoor stream channels was a better measure than biofilm diversity (structure) for identifying experimentally induced stress.

A focus on headwater streams is interesting because the terrestrial and aquatic compartments are most strongly connected at headwater streams with strong expected effects of terrestrial processes for the ecological functioning in the aquatic compartment. Assessments of functional integrity in streams have been done using measurements of ecosystem-level ecological processes including litter decomposition and grazing rates and measurements of stable isotope profiles (e.g. Castela, Ferreira & Graça (2008); Gessner & Chauvet (2002)).

Several studies highlight the importance of allochthonous detritus as an important source of energy for small streams (e.g. Leberfinger, Bohman & Herrmann (2011), Vannote *et al.* (1980); Wallace *et al.*(1997)). However, whereas it has been shown that allochthonous detritus comprises the major source of energy in forested streams (Anderson & Sedell, 1979; Gessner, Chauvet & Dobson, 1999; Mann *et al.*, 1988; Wallace *et al.*, 1997), less attention has been paid to the ecology of open-canopy streams like those draining shrub and tundra catchments (Leberfinger *et al.*, 2011). Open-canopy streams have been generally assumed to mostly depend on autochthonous sources of carbon and energy due to limited terrestrial subsidies (Hagen *et al.*, 2010), but a study by Leberfinger *et al.* (2011) suggested that the abundance and production of leaf-eating aquatic insects may in fact be higher in open-canopy than forested streams because of opportunistic feeding on a more diverse food source. Autochthonous primary production in streams, including the production of algae and macrophytes, is dependent on light and nutrients. Whereas shading is an important limiting factor for autochthonous production in forested streams, nutrient levels are rarely limiting (Greenwood & Rosemond, 2005; Hagen *et al.*, 2010; Webster & Meyer, 1997). Open-canopy streams, depending on geology, topography, vegetation cover and land use, may rely on a more diverse pool of resources including both light and nutrients. Whereas streams flowing through tundra may drain open areas with very scarce vegetation or areas surrounded by dwarf shrubs, herbs and grasses, and thereby may receive much light and experience great variation in temperature, streams flowing through shrubby vegetation may experience shading and a more stable micro-climate. The relative lack of studies in open-canopy streams and the partly contradictory findings suggest that the effects of vegetation cover in alpine habitats on the ecological functioning of headwater streams are still incompletely understood.

Stable isotope analysis (SIA) has become a valuable technique for the examination of trophic interactions and energy pathways by measuring ratios of stable isotopes of elements such as C, N and P. Compared to terrestrial plants, freshwater photosynthetic organisms, such as algae, usually experience depletion of ^{13}C in water resulting in a difference in the stable isotope ratios of allochthonous material entering the stream compared to autochthonous primary producers. The difference in the isotopic ratios among food sources can affect the ratio in the tissue of a consumer through enrichment reflecting fractionation. Stable isotope ratios may hence provide an indication of trophic status. Further, excretion of the lighter ^{14}N isotope results in a 3–5 ‰ enrichment in ^{15}N of consumers relative to their food source providing further valuable information on trophic status (Doucett *et al.,* 1996; Jones *et al.*, 1998; Lancaster & Waldron, 2001; Peterson & Fry, 1987).

The goal of my study was to analyze key ecological processes in headwater streams draining three types of vegetation cover and to assess how the manipulation of snow cover may affect ecological functioning. I therefore measured primary production, grazing rates and leaf decomposition rates, and studied the content and assimilation of carbon and nitrogen by different primary producer and consumer species using stable isotope analysis.

Theoretically, reduction in the snow cover should reduce soil and water temperatures and hence alter key ecological processes in headwater streams draining three types of vegetation cover: forest, shrub and tundra. I hypothesized primary production and grazing rates to be higher in tundra than forested streams (whereas streams draining shrubby catchments should show an intermediate pattern) and the reverse pattern for decomposition rates due to differences in the availability of light and organic matter as suggested by findings by Leberfinger *et al.* (2011). With the expected higher autochthonous production and less allochthonous material available in streams flowing through tundra, I hypothesized stable isotope values to show a depletion of ^{13}C in macroinvertebrate species compared to the same species in streams draining forested and shrubby areas. Further, I expected the caddisfly *Chaetopteryx villosa* to be enriched in ^{15}N due to its feeding habit (both shredder and predator) relative to the other (non-predatory) macro-invertebrate species. Finally, I expected leaves of *Betula* and *Salix*, sampled in the stream (detritus) and in the riparian zone within the site, to have similar isotopic profiles expecting both samples originated from the same area.

2. Materials and methods

2.1 Study area

The present study was conducted in the Dovre Mountains in Central Norway (Fig. 1) from September 2012 to November 2013 following a pilot study in 2011-2012 (Petrin *et al.*, unpubl. data). The streams were located in an area where the mean winter temperature (Dec.-Feb.) was -8.1 °C, and mean winter precipitation was 82 mm from 1961 – 1990 and -7.3 °C and 84 mm from 2001 – 2010 (Fokstugu metrological station, 973 masl., approx. 27 km south-west from the study catchment; data obtained from the Norwegian Meteorological Institute Climate database, elima.no).

The studied streams drained areas of three vegetation cover types; forested sites characterized by birch (*Betula* spp.) trees; shrub sites dominated by willow (*Salix* spp.) species; and tundra sites characterized by graminoids and bryophytes in the bottom layer and the dwarf shrub *Betula nana*. The study sites were located at first and second order streams. The size of the stream substrate particles ranged from gravel to cobbles interspersed with patches of bryophytes and fine sediment. The geology of the catchment comprised calcareous bedrock, dominated by metamorphic phyllite (Sæther *et al.*, 1981). There were no fish at the studied sites.

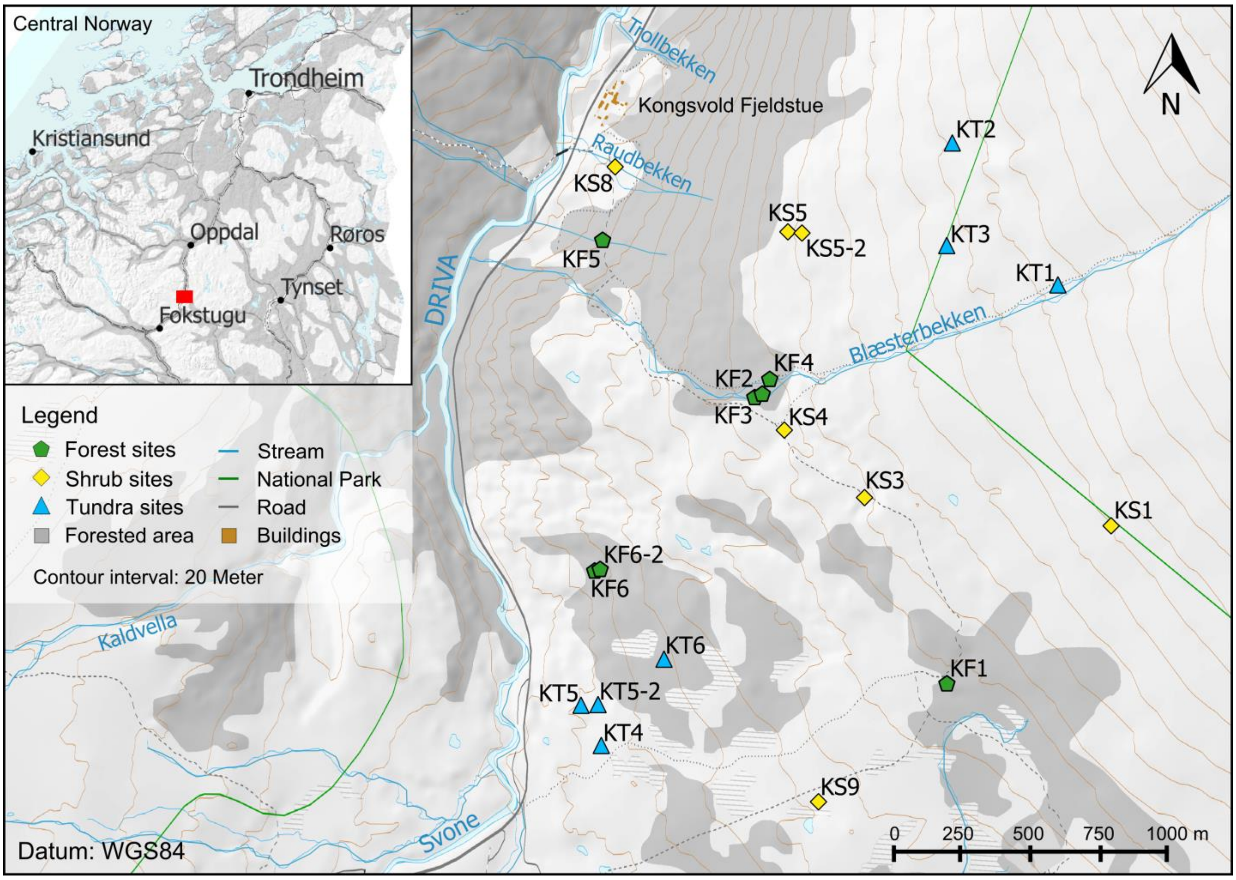

Figure 1. *Map of the study area in the Dovre Mountains in Central Norway marked with a red rectangle in the inset map. The study area borders to the Dovrefjell–Sunndalsfjella National Park to the east and west. Elevation raged from 930 mamsl. (KS8) to 1180 mamsl (KT1).*

2.2 Study design

I considered a total of 21 sites at 18 streams draining catchments of three vegetation cover types: forest, shrub and tundra (Tab. 1). In 15 of the streams, I studied a single site. In each of the three remaining streams, one of each vegetation cover type, I studied one site that was subject to snow depth manipulation during winter, as well as one upstream control site. Each study site comprised a 100 m^2 large plot covering a 20 m long reach of the stream and extending 2.5 m on either side from the middle of the stream. Measurements of ecological functioning including (i) decomposition of leaf litter, (ii) primary production and (iii) grazing rates were done at all sites. Stable isotope analysis of carbon and nitrogen decomposition was done at nine sites. Assessment of vegetation cover and light availability were done at all sites. One of the studied control sites, KS3, was excluded from the study due to delayed snowmelt at that site compared to the other sites and compared to the surrounding area, giving a total of 20 sites. I knowingly left the treatment unreplicated performing a pseudo replication (Hurlbert, 1984), but as proposed by Oksanen (2001), controls were replicated.

Table 1. *Studied sites with treatment (Control (C), Upstream control (UC), Snow depth manipulation (SDM)), measurements (Decomposition rates (DR), Primary production (PP), Grazing rates (GR), Stable isotope analysis (SIA)) and elevation. * KS3 was excluded from the study due to delayed snowmelt at that site compared to the other sites and compared to the surrounding area , giving a total of 20 sites).*

Site	Veg. cover	Treatment	Measurements	Elevation (mamsl.)
KF1	Forest	C	DR, PP, GR	1053
KF2	Forest	C	DR, PP, GR	1049
KF3	Forest	C	DR, PP, GR	1052
KF4	Forest	C	DR, PP, GR	1059
KF5	Forest	C	DR, PP, GR, SIA	946
KF6	Forest	SDM	DR, PP, GR, SIA	954
KF6-2	Forest	UC	DR, PP, GR, SIA	960
KS1	Shrub	C	DR, PP, GR, SIA	1170
KS3*	Shrub	C	DR, PP, GR	1084
KS4	Shrub	C	DR, PP, GR	1056
KS5	Shrub	SDM	DR, PP, GR, SIA	1079
KS5-2	Shrub	UC	DR, PP, GR, SIA	1081
KS8	Shrub	C	DR, PP, GR	934
KS9	Shrub	C	DR, PP, GR	1003
KT1	Tundra	C	DR, PP, GR	1192
KT2	Tundra	C	DR, PP, GR	1161
KT3	Tundra	C	DR, PP, GR	1151
KT4	Tundra	C	DR, PP, GR	956
KT5	Tundra	SDM	DR, PP, GR, SIA	945
KT5-2	Tundra	UC	DR, PP, GR, SIA	945
KT6	Tundra	C	DR, PP, GR, SIA	976

2.3 Snow depth manipulation

Snow depth was manipulated four times at each of the three studied sites (KF6, KT5 and KS5) on 28-30 November 2012, 10 December 2012, 10-12 January 2013 and 08-10 February 2013 (figure 2). The selection of sites to be manipulated was influenced by accessibility during winter. Shovels and brooms were used to remove as much of the snow cover as possible without mechanically damaging the vegetation within each plot. Snow depth manipulations were conducted when the weather forecast predicted a period of at least two-three days with air temperatures below -10 °C following the manipulation. During the first and third manipulation, considerable amounts of snow were removed, while the second manipulation mainly served to maintain the relative snow free condition by removing snow that was blown into the plots, as precipitation was low prior to the second manipulation.

During the last manipulation, considerable amounts of snow were removed from the shrub and forest plots, but less snow needed to be removed from the tundra plot.

2.4 Abiotic variables

Soil and water temperatures were measured at all sites from September 2012 to late May 2013. Water temperature was measured at the bottom of the stream, and soil temperatures at a depth of 1 and 10 cm within 1 m from the stream using temperature data loggers (HOBO Pendant Temperature/Light Data Logger, Onset Computer Corp.) logging at an interval of 10 minutes at 18 sites and (HOBO Onset temperature logger) with an interval of 4 hours at the upstream control sites. Water temperature was recorded during summer to correct for possible temperature effects on leaf litter decomposition, primary production and grazing rates.

Water samples were collected 1-5 m downstream from each plot in September 2012 and May 2013. Chemical analysis of the water samples that was conducted during the pilot study showed that the water at the study sites was generally circumneutral (pH: 7.1-7.8) reflecting the good buffering capacity of the water (0.348 – 1.274 mmol*l^{-1}) due to the underlying calcareous bedrock. Also, chemical analysis suggested a lack of conspicuous levels of important chemical variables including aluminum levels that are known to be important for many freshwater organisms (Herrmann, 2001). The measurements from the pilot study indicated low aluminum concentrations at our study sites (4.7-80.1 µg Al/L), that were below the known toxic range for benthic macroinvertebrates (Petrin *et al.*, 2007). Only selected water chemistry variables were therefore measured in the scope of the present study, including pH, alkalinity and total organic carbon (TOC) concentrations. All chemical measurements were conducted at Trondheim kommune Analysesenteret, Trondheim, Norway.

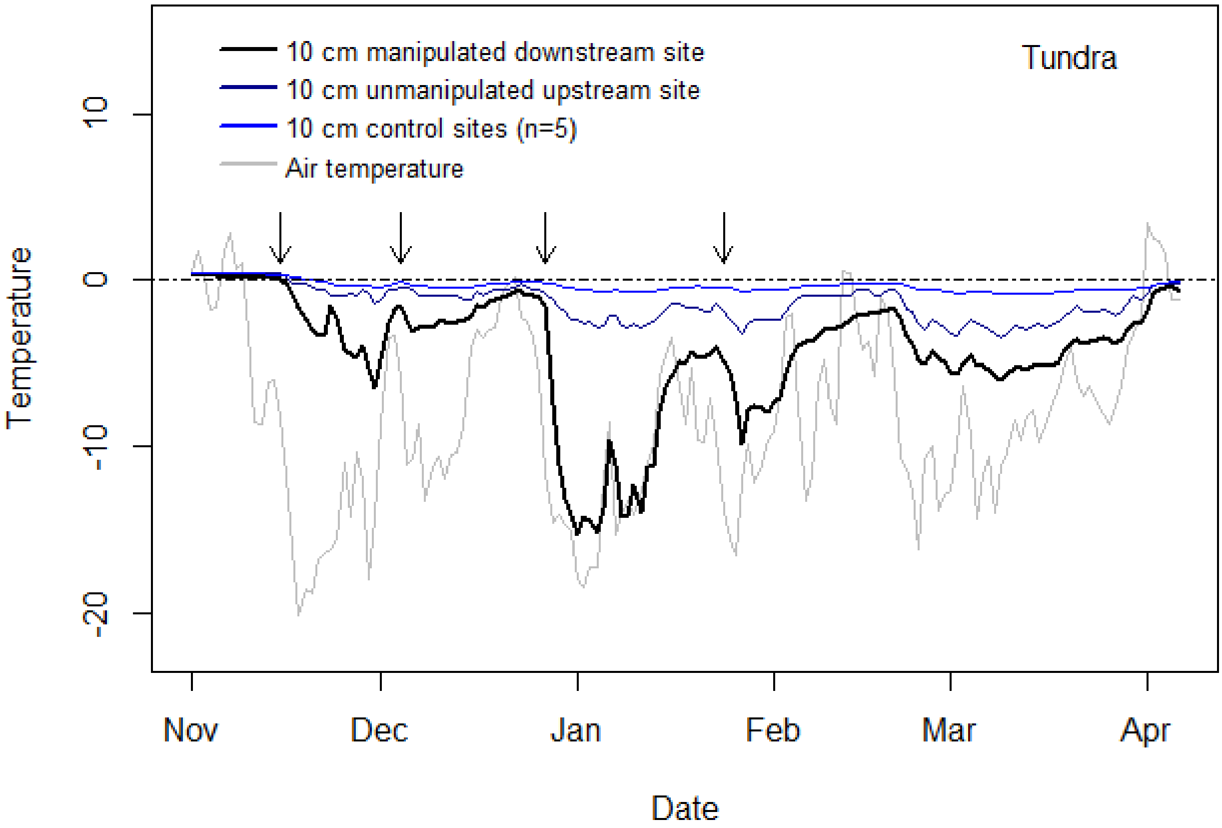

Figure 2. *The effect of snow depth manipulation on temperature at the tundra plot KT5. The thick black line shows data from the temperature logger in the soil placed at a depth of 10 cm at KT5, while the thin blue line shows data from a temperature logger at the same depth at the unmanipulated upstream control site. The grey line represents the air temperature in the same period. The timing of the four snow depth manipulations is shown by the black arrows.*

2.5 Measurements of ecological functioning

2.5.1. Decomposition of leaf litter

Decomposition rates of leaf litter were measured at twenty-one sites to assess microbial decomposition and decomposition due to consumption by benthic macro invertebrates. I used Gray Alder (*Alnus incana)* leaves that were enclosed in mesh bags of two mesh sizes (500 µm and 12 mm). The measurements were implemented as outlined in Benfield (1996). In short, leaves were collected from the same stand of alder trees prior to abscission in September 2012. All mesh bags were filled with 4±0.05 g of air-dried leaves. A total of ten bags, five of each mesh type, were attached to the streambed at each site in June 2013 and in September 2013. The mesh bags were exposed in pairs including one coarse and one fine mesh bag (Figure 3) for a period of 35-38 days. Control mesh bags were used to assess handling losses of leaf material in the period between weighing and attachment to the streambed, and leaching following exposure to the stream environment. Aquatic insects that were found inside the retrieved mesh bags were enumerated and identified to the lowest possible taxonomic level, usually species, and classified according to functional feeding groups following (Malmqvist, Nilsson & Svensson, 1978; Merritt & Cummins, 1996; Petrin, 2011). The remaining alder leaves were dried, weighed, ashed for 4.5 hours at 550°C and reweighed to determine ash free dry mass (AFDM) and account for attached mineral particles.

Decomposition rates were determined by calculating the breakdown rate coefficient k,

$$Breakdown\, rate, k = \frac{\log\left(\frac{AFDM}{iDM_c}\right)}{t_{stream}}$$

where $AFDM$ is the ash free dry mass, iDM_c is the initial dry mass available for decomposition controlled for handling losses and leaching, and t_{stream} is the time of exposure in the stream in days. Possible temperature effects were controlled for by employing metabolic theory (Brown *et al.*, 2004).

Figure 3. *One pair of fine and coarse mesh bags placed in one of the studied streams.*

2.5.2. Measurements of Chlorophyll a content.

Algal primary production and grazing pressure were quantified by measuring chlorophyll a concentrations on unglazed ceramic tiles (surface area, 0.01 m^2) that were placed in pairs onto the streambed for 82 days in June-August 2013 (Lamberti (1996), Lamberti and Resh (1983)). Five pairs of tiles were placed at twenty sites. All vertical sides of one tile of each pair were coated with petroleum jelly to deter crawling grazers, while the other tile was left untreated (Figure 4, Hladyz *et al.* (2011)). At the end of the exposure period, the periphyton on each tile was collected with a spatula onto a GF/C-filter and stored on ice in the dark until drying. The material was then frozen at -47°C for 12 hours to break the cell walls. Pigment extraction was done by adding ethanol as solvent, and the samples were then incubated at 4°C for 36 hours prior to spectrophotometric measurements. Each solution was centrifuged at 13.000 rpm for 10 min, and the supernatant was used to measure absorbance at 665 nm and 750 nm (1 cm cuvettes; the solvent was used as blank) using a PerkinElmer Lambda 40 Spectrometer. For further details, see Hladyz *et al.* (2011), Lamberti and Resh (1983) and Jespersen and Christoffersen (1987). The amount of Chlorophyll a [g] that was sampled from each tile was determined as

$$m_{Chl\ a}\ (g) = \frac{(Abs_{665} - Abs_{750}) * V_e}{Abs_c^{-1} \times d^{-1}}$$

where Abs_{665} was the absorbance of the sample at 665 nm (peak absorbance of Chlorophyll a), Abs_{750} was the absorbance of the sample at 750 nm (control for turbidity), V_e was the volume of the extract, Abs_c^{-1} was the absorption coefficient, 83.4 l g^{-1} cm^{-1} for ethanol (Wintermans & de Mots, 1965), and d^{-1} was the length of the light path through the cuvette (1 cm).

Figure 4. *Pair of unglazed ceramic tiles placed onto the steambed in one of the studied streams. One tile (to the right in the picture) had the vertical edges covered with petroleum jelly to deter crawling grazers.*

2.5.3 Stable isotope analysis

Analysis of stable isotopes was done by using materials that were collected in September 2012 and in late May 2013 at nine sites in six streams (KF5, KF6, KF6-2, KS1, KS5, KS5-2 and KT6) to assess consumer-resource interactions. The sampled material included birch (*Betula* spp.) leaves and willow (*Salix* spp.) leaves that were collected on the ground at each site – and leaves and aquatic bryophytes that were collected in the stream. No periphyton was collected due to the scarcity of the material in most streams. Invertebrate taxa that were sampled for the determination of isotope profiles included *Chaetopteryx villosa* (Fabricius), *Nemurella pictetii* (Klapálek), *Baetis rhodani* (Pictet), *Leuctra nigra* (Olivier) and *Amphinemura sulcicollis* (Stephens), which dominated the shredder and grazer guilds in most streams. Invertebrate samples were preserved in ethanol (approximately 80%) at the time of collection, sorted and classified to species level in the laboratory and subsequently dried at 60°C prior to weighing and stable isotope analysis. Materials were weighed using a microbalance (Mettler Toledo XP2U Ultra microbalance) (amount of material, leaves and bryophytes: 0.3-0.4 mg, invertebrates: 0.5 mg, resolution: 1.0 µg). The stable isotope analysis was conducted at Risø National Laboratory for Sustainable Energy at the Technical University of Denmark (DTU), Roskilde, Denmark. The total content of carbon and nitrogen and isotopic ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were measured by a mass spectrometer (Finnigan MAT Delta PLUS, Thermo Scientific, Bremen, Germany) coupled to an elemental analyzer (CE 1110, Thermo Electron, Milan, Italy). Pure gases of CO_2 and N_2 were used as working standards in the isotope ratio analysis of ^{13}C and ^{15}N, respectively. Isotope values, the relative difference in the isotope ratios between the samples and the standard, are reported as delta (δ)

$$\delta X\ (‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where δX is $\delta^{13}C$ or $\delta^{15}N$, respectively, R is the isotope ratio of heavy to light isotopes in the sample ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) and relative to the standard (Peterson & Fry, 1987).

2.6 Mapping of vegetation

The study streams were classified into three types of vegetation cover prior to sampling. Mapping of the vegetation was conducted in August 2012 at eighteen sites; the vegetation at the three upstream control sites was mapped one year later, in late August 2013. Mapping was conducted using five 5 m line-transects that were placed perpendicularly to the stream and equidistantly spaced at 2 m intervals starting at the bottom of each plot. The mapping was conducted at two scales: fine and coarse. At the coarse scale, the dominating component at every meter was determined in four layers: tree layer, shrub layer, field layer and ground layer (in total, five components in each layer along each transect). Ten vegetation and non-vegetation classes were used in total (see Appendix 1). I also recorded the average height of the tree and shrub layers. Mapping at the fine scale was done using a modified point-intercept method recording the components at 10 cm intervals (in total, 50 components along each transect) using the first-hit approach, where the first class to intercept each point starting from above was recorded. The height of the tree layer was estimated for (i) trees in the area, and if present, (ii) trees in the plot.

2.7 Measurement of shading using the Shade Proportion Index (SPI)

To measure the amount of light entering the streams, I used a modified method for measuring and calculating the Leaf Area Index (LAI), including not only the hemispherical area covered by the surrounding and over-head vegetation, but also by local topographical features. Since the focus of this modified index was to quantify the amount of shading of a given stream, the index will hereafter be referred to as the Shade Proportion Index (SPI). Measurements were conducted in the end of August 2013 by capturing hemispherical photographs at each site except for KS3. Two hemispherical photographs (hereafter hemiphotos) were taken above the stream at five spots at each site, on par with the five transects used in the mapping of the vegetation. One photo was positioned directly above the stream at the height of the surrounding ground, and the second at the height of the top of the shrub layer. Seven spots (at the sites KT5, KT6 and KF5) had no shrub layer; therefore, no hemiphotos were taken at these spots, giving a total of 193 photographs. The hemiphotos had a 3872 x 2048 pixel resolution and were captured using a Nikon D80 camera with a Nikkor 10.5mm f/2.8G ED DX fisheye lens. Calculations of SPI followed the calculations of LAI and were done using the image processing software ImageJ (Abramoff, 2004). Binary B/W pictures were therefore produced with white pixels representing sky and black pixels vegetation and other topographical features by manually adjusting the threshold in each hemiphoto and correcting remaining on-ground light areas (e.g. lichens, bare ground etc.) by painting them black (see Appendix 2 for image examples). The radial profile plugin (extension of ImageJ by Baggethun P.) was then used to produce plots that could be compared and used to calculate the index.

In this study, categorization of streams into vegetation cover was done using two different techniques; i) vegetation mapping at fine and coarse scales, and ii) measurements of shading using the Shade Proportion Index (SPI). The two techniques produced three slightly different types of classifications. In short, whereas fine scale vegetation mapping provided a qualitative measurement of non-woody vascular plants (but missed out on the structural importance of higher trees), the mapping at the coarse scale was dominated by woody vegetation (Appendix 1). The last technique, a structural measurement of shading, yielded no information on species composition, but solely quantified the amount of light entering the stream. Further, measurement of shading, conducted directly above the water surface, distributed the sites continuously according to structural attributes. Due to these differences and the focus of the present study including measurements of primary production and decomposition of leaf litter, which depended on light and the input of leaf detritus, the choice fell on SPI as the preferred predictor variable.

2.8 Statistical analyses

The effects on decomposition were analyzed using linear mixed effects models with treatment (snow depth manipulation/control) as a fixed effect and shading (SPI) at the bottom layer as a covariate. Site and season (autumn/spring) were treated as random factors to account for between site and seasonal variation. Decomposition by microbes was analyzed by directly using the breakdown rate *k* from the fine mesh bags, while decomposition by macro invertebrates was analyzed by using the difference between the breakdown rate from coarse mesh bags and fine mesh bags. Reflecting the study design, three models were used to analyze the data by analysis of covariance (ANCOVA). The first model compared the snow depth manipulated sites to control sites, the second model the manipulated sites to upstream control sites, and finally, upstream controls were compared to control sites at other streams providing valuable information on the comparability of manipulated sites and control sites (due to the lack of replicate manipulated sites). A two-sample Kolmogorov–Smirnov (K-S) test (reported by the *D* statistics) was conducted to compare the variance of isotopic delta values of carbon and nitrogen of the different food sources and macroinvertebrate species. All statistical analyses were performed in R, version 3.1.0 (R Core Team, 2014), using the R packages nlme, lattice, xts and lubridate (Grolemund & Hadley, 2011; Ryan & Ulrich, 2014; R Core Team, 2014; Sarkar, 2008; Pinheiro *et al.*, 2014). All tests were performed with a probability for type I errors of 5 %.

3. Results

3.1 Snow depth manipulation

Snow depth manipulation and the resulting reduction in snow cover caused strongly contrasting winter soil and water temperature regimes (Fig. 6). Minimum winter temperatures at the manipulated sites ranged from –15.3 °C at the tundra to –7.2 °C at the shrub site at a soil depth of 10 cm, while the mean minimum temperatures at the control sites ranged from –1.0 °C at tundra to –0.4 °C at forest sites. The temperatures at the upstream control sites did not differ from the temperatures at the remaining control sites (all $p > 0.1$) (Fig. 6). Minimum water temperatures at the manipulated sites ranged from –8.3 °C at the tundra to –3.5 at the forest site (Fig. 6). Temporal effects of the manipulation of the snow cover included a greater impact on soil temperatures later during the winter (Appendix 5).

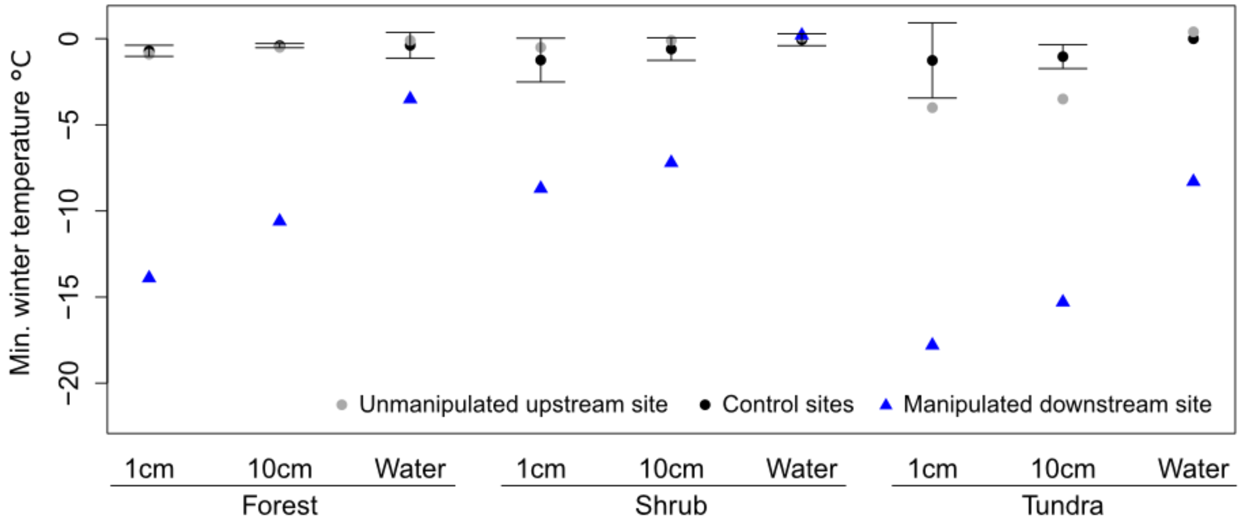

Figure 6. *Minimum soil (depth: 1 and 10 cm) and water temperatures at sites of three vegetation cover types during winter. Data for one manipulated and for one upstream control site per vegetation cover type and for 5, 4 and 5 forest, shrub and tundra control sites, respectively. See Appendix 4 for further details.*

3.2. Decomposition of leaf litter

The rates of leaf litter decomposition were 355 % higher in spring than autumn. The microbial contribution to leaf litter decomposition (16.8 %) was modest compared to shredding, i.e. shredders contributed most to leaf mass loss across all vegetation cover types (Fig. 7). Decomposition by shredders was similar regardless of shading in the autumn.

Decomposition by microbial agents did not differ significantly with snow depth manipulation or shading when comparing manipulated downstream sites to control sites (all $p > 0.1$). However, when manipulated sites were compared to upstream control sites, decomposition decreased (by 37 % at forest compared to tundra sites) with increasing shading (ANCOVA SPI $F_{1,7} = 9.612$, $p = 0.012$, SDM $F_{1,7} = 0.614$, $p = 0.459$, SDM × SPI $F_{1,7} = 0.007$, $p = 0.936$). Upstream control sites and control sites did not differ for either variables (all $p > 0.1$).

Decomposition by shredders showed the opposite trend in tundra and forest when comparing snow depth manipulated sites to control sites (Fig. 7, Tab. 2). Whereas decomposition in tundra was found to be lower at downstream manipulated sites (10 % of control), the decomposition in forest was higher at control sites (595 % of control). However, it remained unclear whether the lower decomposition rates were a result of the snow depth manipulation or increased shading. No difference was found among shrub sites.

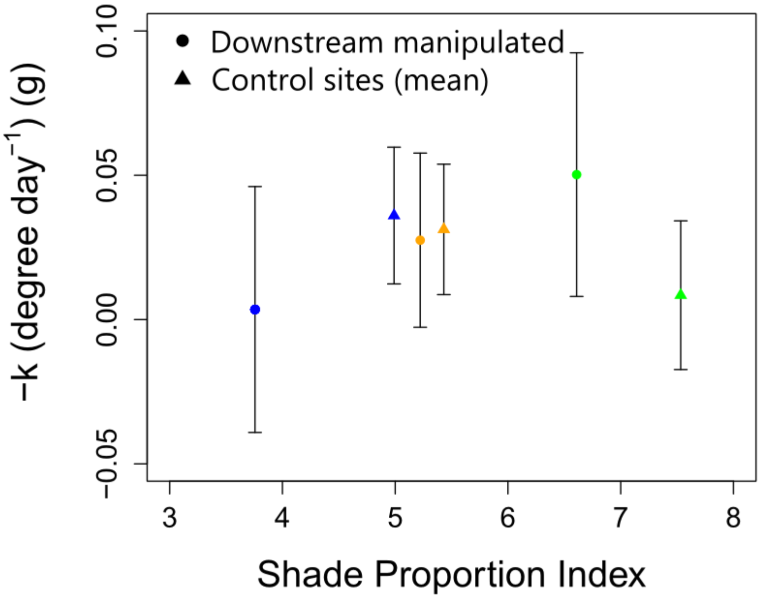

Figure 7. *Decomposition of leaf-litter by shredders comparing downstream manipulated sites (3 sites) to control sites (14 sites) (mean ± SE). Note: Blue, orange and green denotes tundra, shrub and forest sites.*

Further, decomposition was found to be lower at manipulated sites when compared to upstream control sites (Fig. 8a, Tab. 2). The reduction was caused by increased shading. No effect was found for snow depth manipulation. Comparing the two levels of controls, control sites to upstream control sites, showed a marginally insignificant effect of shading (Fig. 8b, Tab. 2) and no difference between the stream, where the manipulation was done and the remaining control streams.

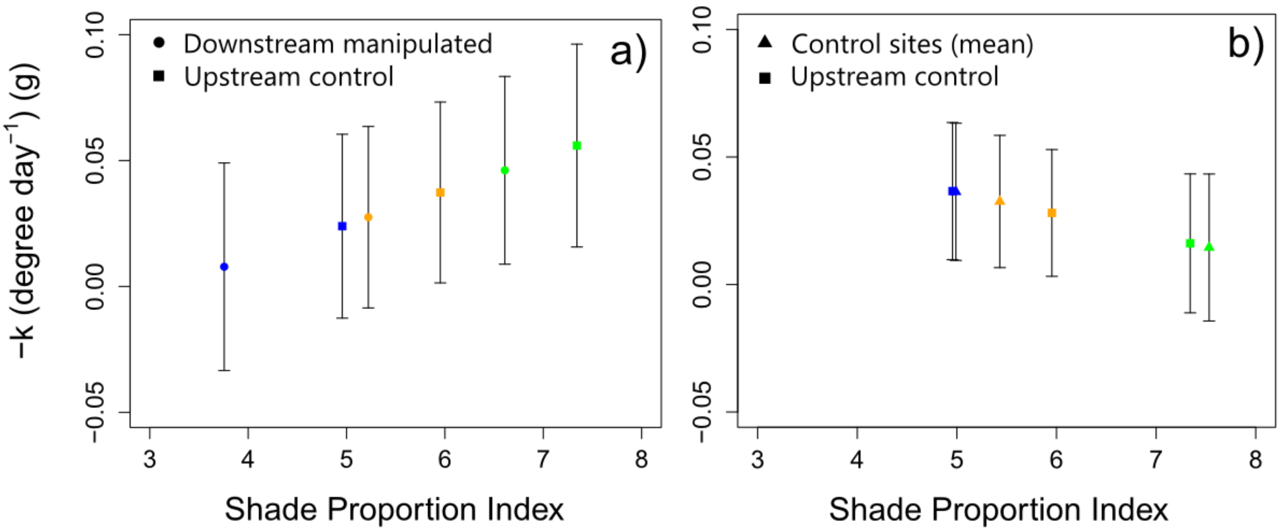

Figure 8. *Decomposition of leaf-litter by shredders comparing a) downstream manipulated sites (3 sites) to upstream control sites (3 sites) and b) upstream control sites (3 sites) to control sites (14 sites) (mean ± SE). Note: Blue, orange and green denotes tundra, shrub and forest sites.*

Table 2. *Effects of snow depth manipulation, vegetation cover (SPI) and their interactions on decomposition rates of leaf litter (k) by shredders (ANCOVA). F; conditional F-test statistics with numerator and denominator degrees of freedom; control sites, control sites and unmanipulated control streams.*

Model, predictor variables	**Test statistic**	***p* value**
Manipulated downstream sites vs. control sites		
Snow depth manipulation (SDM)	$F_{1,27} = 0.053$	0.820
Shading (SPI)	$F_{1,27} = 2.512$	0.125
SDM × SPI	$F_{1,27} = 5.595$	0.026
Manipulated downstream sites vs. upstream control sites		
Snow depth manipulation (SDM)	$F_{1,7} = 0.880$	0.380
Shading (SPI)	$F_{1,7} = 5.864$	0.039
SDM × SPI	$F_{1,7} = 0.353$	0.571
Upstream control sites vs. control sites		
Upstream control (US)	$F_{1,27} = 1.205$	0.282
Shading (SPI)	$F_{1,27} = 3.567$	0.069
US × SPI	$F_{1,27} = 2.171$	0.152

3.3. Measurements of the chlorophyll a content

Algal primary production was quantified at five out of twenty sites due to a summer drought exposing tiles to the air and the sun during long periods at the remaining fifteen sites (Fig. 10). Algal production at the five streams where chlorophyll a concentrations could be measured ranged from as little as $1.4*10^{-6}$ to $7.5\ 10^{-5}$ g (dry mass) m^{-2} degree day^{-1} in agreement with the observed relative lack of periphyton in most streams. Due to the lack of data from most streams, including two out of three manipulated streams, I was unable to statistically test if there was an effect of snow depth manipulation on algal primary production.

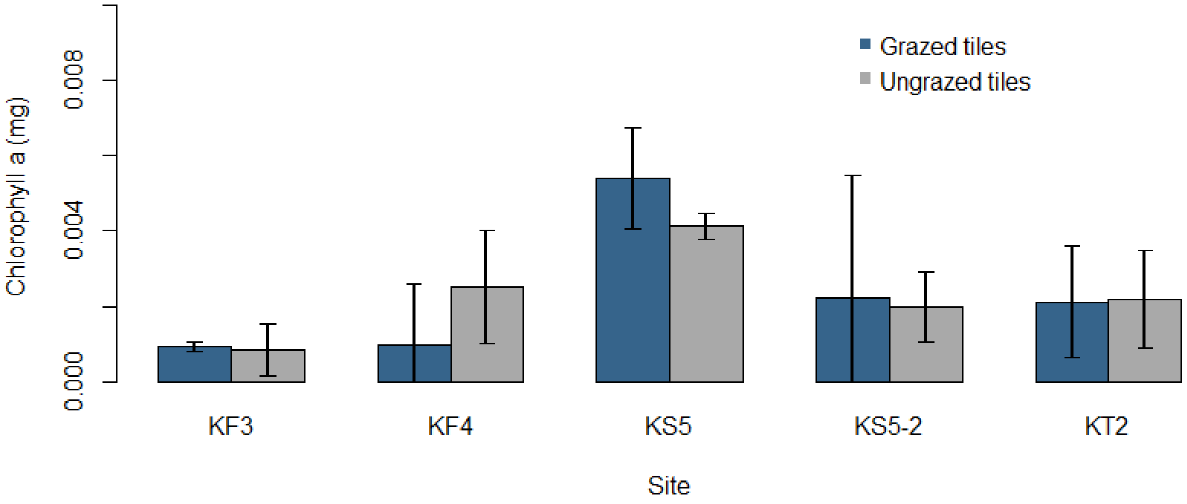

Figure 10. *Measured accrued algal primary production (dry mass) on grazed and ungrazed ceramic tiles at five sites after 82 days.*

3.4. Stable isotope analysis

The isotope profiles of potential food sources and macro invertebrate species are illustrated using isotopic bi-plots presenting mean values of $\delta^{13}C$ and $\delta^{15}N$ and the variation within each population (Fig. 11 and Fig. 12). The data for aquatic mosses were removed from the bi-plots due to large variance (see Appendix 8 for figures including mosses). No relative change in the stable isotope profiles that was caused by vegetation cover or snow depth manipulation was observed. Visible clustering of samples of *Betula* and *Salix* leaves was supported by pairwise comparisons of isotopic values (Appendix 7). Macro invertebrate species differed in their isotopic profiles and were ordered according to their feeding habits (Fig 11 and 12, Appendix 7). Further analysis of the relative proportions of the sampled food sources that were consumed by the tested macroinvertebrates was not conducted due to a lack of diversity in the isotopic profiles of the sampled food sources.

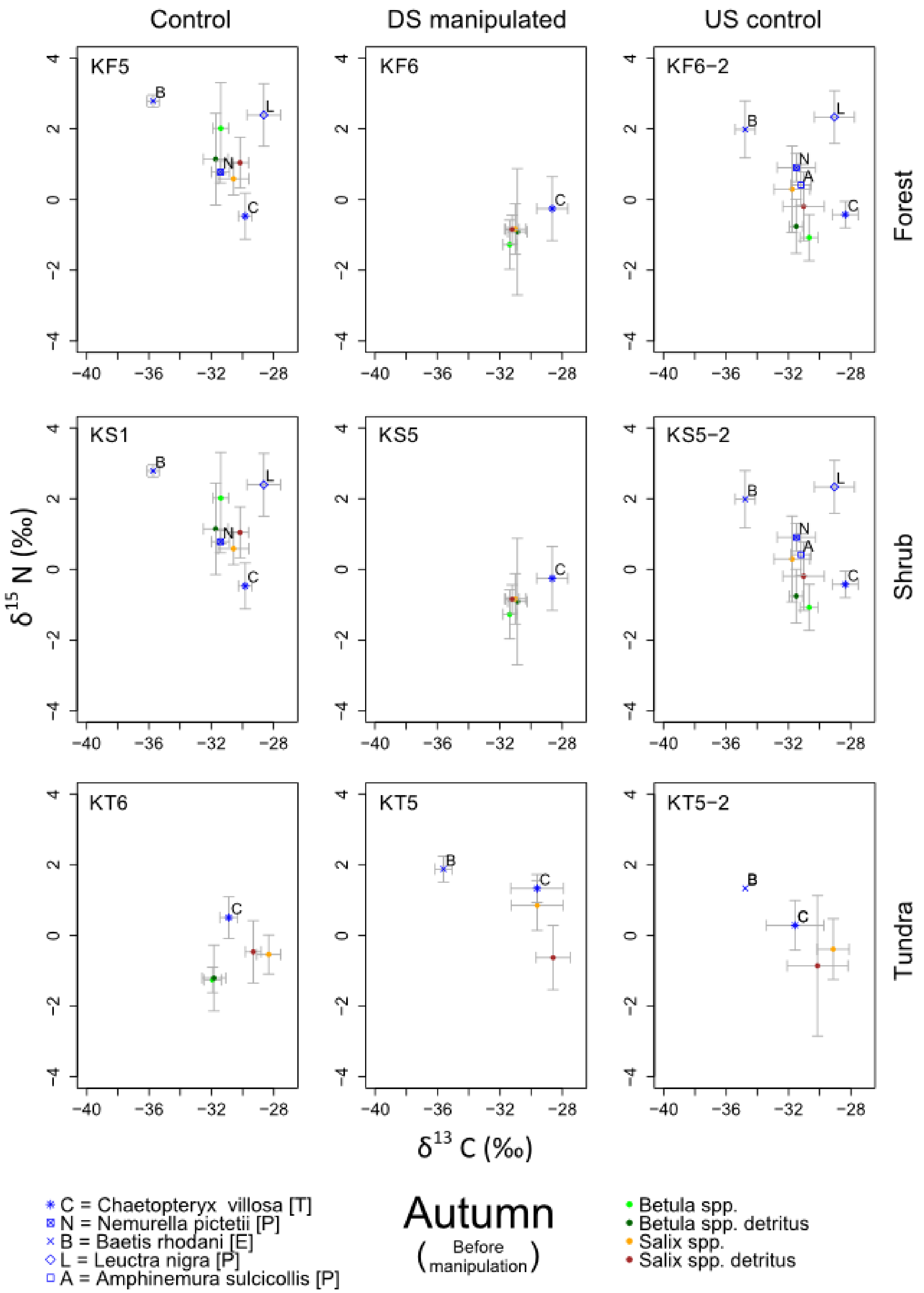

***Figure 11**. Isotopic bi-plots of mean ±SD $\delta^{13}C$ and mean ±SD $\delta^{15}N$ values of potential food sources and macro invertebrate species at nine sites in the autumn (prior to the manipulation of the snow depth).*

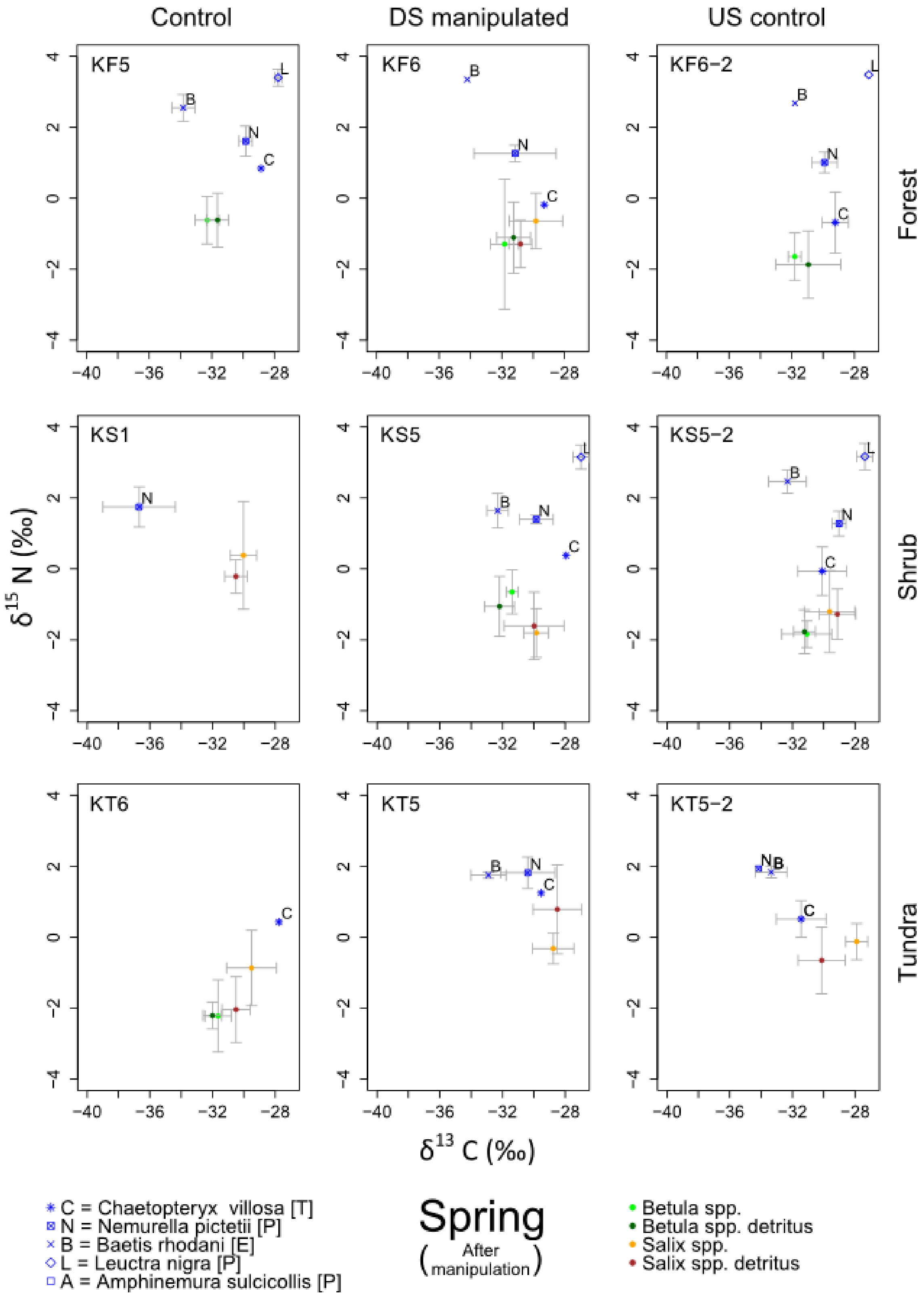

Figure 12. *Isotopic bi-plots of mean ±SD* $\delta^{13}C$ *and mean ±SD* $\delta^{15}N$ *values of potential food sources and macro invertebrate species at nine sites in the spring (following manipulation of the snow depth).*

3.5. Site and habitat characteristics

In both autumn (2012) and spring (2013), the water was circumneutral (pH 7.0–8.0) and well buffered (0.2-1.274 mmol*L^{-1}) at all sites. The amount of total organic carbon (TOC) ranged from 0.8 – 5.7 mg C L^{-1} (Appendix 2). The water in forested streams was colder (mean 6.7 °C, SD 0.75) than at the remaining sites (tundra 7.7 °C, SD 0.85, and shrub 7.4 °C, SD 0.43), and the forested sites were more shaded (Shade proportion index (SPI); mean 7.4, SD 0.8 vs. shrub: mean 5.5, SD 0.5 and tundra: 4.8, SD 0.5).

4. Discussion

The findings of the present study demonstrated a reduction of soil temperatures following manipulation of the snow depth across all vegetation cover types. However, the results could not clearly show how the manipulation affected decomposition of leaf-litter. Decomposition rates were, as predicted, affected by vegetation cover, either alone or with snow depth manipulation, yet in a somewhat unexpected manner. Further, manipulation did not alter the $\delta^{13}C$ and $\delta^{15}N$ stable isotope values of macroinvertebrate species. Manipulation and vegetation cover effects could not be assessed for algal primary production and grazing rates due to a lack of data, yet my results suggested that primary production and consumption of autochthonous resources was of minor importance.

4.1 Leaf litter decomposition

Snow depth manipulation caused soil freezing and subzero water temperatures in forest and tundra streams. In catchment with these vegetation cover types, I found decomposition rates at manipulated sites to differ from control sites. Yet, whereas decomposition at the snow depth manipulated site in the forested stream was higher compared to control sites, the opposite was found for the tundra stream. No change was found at the shrubby site. Manipulated sites in both tundra and forest experienced less shading than their respective control sites. Given the reported importance of allochthonous input of leaf-litter (Vannote *et al.* (1980); Wallace *et al.* (1997); Leberfinger *et al.* (2011)), limited access of detritus may affect macroinvertebrate species composition. Hence, the number of shredders at the manipulated sites may have been lower than at the control sites. It is also possible that the measured subzero water temperatures may have caused a significant reduction of shredder abundance and thereby reduced the rate of decomposition. But neither shading nor manipulation of snow depth was found to significantly affect decomposition rates alone. At forested sites, decomposition rates were higher at the snow depth manipulated site. If leaf-litter flooded the pool of resources in the forested streams, then the macroinvertebrate guild may not be limited by leaf-litter. Hence, the leaf-bags used to assess decomposition rates may have been in excess indicating that the availability of leaf litter may not have been the only factor controlling decomposition rates. Observations of leaf litter lost during the autumn and still present in the spring at forested sites supports this explanation. However, the manipulated forested site was also subject to bottom freezing. Most macroinvertebrates overwintered as nymphs or larvae. As long as temperatures are above freezing, macroinvertebrates may remain active during winter, or migrate to habitats that will freeze in early winter (Lencioni, 2004). The temperatures at the tundra (–8.3°C) and forest (–3.5°C) sites were below what has been reported to be lethal (Oswood, Miller & Irons, 1991). However, since the studied streams were lotic (with flowing water), it is possible that not all parts of the streams in the tundra and forest were frozen, providing sheltered habitats that do not freeze (Olsson (1981); Sinclair, Addo-Bediako & Chown (2003)). Hence, the manipulated forested site may have provided better or additional above zero refugia (given the higher minimum temperature and stream morphology) with more shredders surviving and the resulting higher rate of decomposition.

An extra upstream control was added to the manipulated streams draining the three different vegetation covers in order to examine whether the streams that were manipulated differed from control streams of the same vegetation cover type. In contrast to the possible effects of snow depth manipulation that I found comparing manipulated sites to control sites, only shading was shown to affect decomposition comparing downstream manipulated sites to upstream control sites. Comparison of the two levels of control produced a marginally insignificant change in decomposition with increasing shading, however in the opposite direction than above. Ideally, the upstream control sites should have had the same structural attributes (i.e. shading) as the manipulated site and control sites within each vegetation cover type. Whereas the control sites and upstream controls were quite similar, all three manipulated sites were less shaded than both levels of controls.

Less shading of manipulated sites may have affected the results. E.g. if the reduced shading caused a complete or partial lack of allochthonous material at the tundra site, then the density of shredders may have been too low to allow for the efficient decomposition of the leaf litter in the mesh bags. In addition, the scarcity of periphyton may have limited the densities of potentially opportunistic macroinvertebrates that may also have functioned as shredders. At the manipulated forested site, decomposition rates were higher than at the control sites, even when considering the lower degree of shading. If the amount of leaf-litter was less at the manipulated site than at the control sites, then the experimental mesh bags with leaves could have been heavier exploited at the manipulated site resulting in higher rates of decomposition, given that the shredders survived the manipulation. However, a study by Aspaas (2014) showed that the abundance of shredders in fact was higher at the manipulated site and the upstream control site compared to the other controls.

4.2 Stream primary production and grazing rates

Little stream primary production, measured as periphyton growth on clay tiles, was documented in the present study. Visual observations of growth (or rather the lack thereof) on natural sediments in the stream channels were consistent with the results. Most streams experienced a long dry period during the summer. The number of replicates that was considered was insufficient to assess whether primary production or grazing rates were altered by manipulation of the snow depth or vegetation cover. The results and observations may, if the studied year was no exceptional event, indicate that the macroinvertebrate assemblages in the study area may comprise opportunistic consumers including collectors that mainly rely on e.g. fine particulate organic matter (FPOM) rather than periphyton.

The scarcity of primary production was surprising given the measured physical properties of the studied streams (i.e. water temperature, nutrient content and access to light). However disturbance events, such as flooding and changes in temperature, have been shown to affect algal production and increase abrasion by mineral particles (e.g. Biggs and Close (1989)). Even though the discharge levels of most of the studied streams were limited, such floods were observed after heavy rainfalls. According to Lake (2003), droughts may overall negatively affect primary production. However, droughts may also result in an increase in primary production in isolated pools that are temporarily disconnected for the duration of the flood. One of the studied streams (KT3) was, found to be disconnected into such pools during summer, yet primary production levels were below the limit of detection.

4.3 Stable isotope analysis

Stable isotope analysis demonstrated no isotopic difference between terrestrial food sources of the same genus, whether collected in the stream or next to the stream (Appendix 7). Further, it demonstrated only small isotopic differences between birch and willow leaf litter (Appendix 7). I chose to exclude data for aquatic mosses for two reasons; i) greater variation in the measured isotopic values of carbon than expected, and ii) I did not expect bryophytes to be part of the diet of the macroinvertebrates in question (Dangles, 2002). The unexpected variation of carbon isotopic values of bryophytes might have been the result of sampling not only living material, but also dead parts and perhaps fine particulate organic matter attached between the leaves. The different components probably had different stable isotope profiles, which increased the variation in the stable isotope signatures. In total, five macroinvertebrate species were sampled in the study. However, not all species were present at all sites in both autumn and spring. The caddisfly *Chaetopteryx villosa* and the stonefly *Nemurella pictetii* were present in most streams as expected from their feeding habit (Malmqvist, Nilsson & Svensson (1978); Merritt & Cummins (1996); Petrin (2011)). The stonefly *Amphinemura sulcicollis* was only found at two sites in the autumn (KF6-2 and KS5-2) and the general number of individuals of other species was often less than seven at each site. Hence, the sample size of collected macroinvertebrates was minimal. From the results, I concluded that the sampled food sources could not explain the variation in the stable isotope profiles that was observed in macroinvertebrate species, indicating that I was unable to sample the full range of potential food sources in these streams. Hence, no analysis of the macroinvertebrates' potential dietary spectrum was done. Such a comparison would also require that the chosen species were present at most, or preferably each site, and during each season in sufficient numbers. The criteria were not fulfilled in this study. Overall, I found no relative changes in isotopic profiles depending on vegetation cover or snow depth manipulation.

4.4 Study design

The study comprised several novel components including the manipulation of the snow depth to study ecological functioning in fluvial ecosystems that drain catchments of different vegetation cover types including open-canopy streams. Also, I assessed possible interactions of snow depth manipulation and vegetation cover at the forest-tundra transition. The long term effects of vegetation cover on measured ecological processes were described through well replicated control sites of all vegetation types. Further, I consciously performed a pseudoreplication of the snow depth manipulation because a cross- factorial experimental design with nine snow depth-manipulated sites (three of each vegetation cover type) was logistically unfeasible. However, Oksanen (2001) claimed that under certain circumstances, such as when working with large-scale ecosystems, proper replication of the treatments may be practically impossible, and proposed different alternatives in order to handle the logistical and economic problems of such experiments. One alternative was to replicate the control, but leave the treatment unreplicated, which is what I did. In addition, I included control sites upstream from the manipulated sites strengthening the results. The effect of the snow depth manipulation was short lived compared to the effect of the vegetation cover. Hence, comparing the strengths of the effects of the two variables was challenging.

The isotopic values of macro invertebrates in this study were obtained using a whole body approach, which is commonly used when dealing with small insects. Here, the stomach is not removed from the specimens by dissection. Hence, the resulting isotopic values are modified by ingested food material. Long term effects may be caused by vegetation cover and short term effects by snow depth manipulation. Up- or downstream colonization or emergence of insects that may remain in an inactive life-stage in unfrozen sediments may have masked the effect of the manipulation.

Despite of the challenges, I believe that the study design with multiple levels of control and comprehensive measurements of abiotic and biotic factors facilitated an assessment of stream functional integrity concerning the effects of vegetation cover and the reduction of the snow depth.

4.5 Winter ecology

The late winter season (February-April) is known to be an important ecological period in aquatic and especially terrestrial systems affecting e.g. seedling growth and survival (Repo *et al.*, 2005). My data, especially from the tundra, show that snow depth manipulation early during the winter, combined with sparse precipitation and cold air temperatures in this period, reduced soil temperatures in this period. Hence, a possibly altered soil biochemistry (e.g. Bölter *et al.* (2005); Clein & Schimel (1995)) may have damaged roots and started a process that may have affected understory plant cover and community composition in the long term (Kreyling *et al.*, 2012). But even though manipulated sites remained colder than control sites for long periods after the last manipulation, temperatures rose from around zero at about the same time at all sites with no apparent delay (Appendix 5). No measurements of snow depth were recorded after the last manipulation, therefore it is unknown whether the snow cover ended earlier at manipulated sites causing greater stress to the vegetation at manipulated sites than at control sites. Further, no effect of increased run-off was measured at manipulated sites (Appendix 3). Four mechanical reductions of the snow cover depth reduced soil and water temperatures in all manipulated streams, as expected (Appendix 4 & 5). The greatest reduction in temperature was found in tundra, the least in shrub streams. Further, the tundra site had 140 days with sub-zero water temperatures, while the forest site had 122 days of sub-zero water temperatures (Appendix 4). The shrub site and the upstream control sites did not bottom freeze. Mechanical removal of snow required gentle handling of the vegetation. Hence, the measured difference in the effect of the manipulation might reflect whether the density of the vegetation allowed for an effective removal of snow. The findings may also have natural causes (Kreyling *et al.* (2012); Liston *et al.* (2002)), given that snow tends to accumulate better at shrub sites. Snow may then provide better insulation and result in smaller reductions of soil temperatures.

4.6 Implications and perspectives

If the distribution of vegetation cover changes as suggested by models of climate change and if land use in alpine areas is altered, the proportion of tundra will decrease in favor of shrub and forest vegetation (Haei *et al.* (2010); IPCC (2007)). Additionally, as shown by the present study, a less stable snow cover may increase soil frost and possibly alter benthic invertebrate communities through bottom freezing of the stream channels. Based on my findings, I would predict ecological functioning, especially decomposition, to be reduced in tundra and possibly in forested streams. I would expect no change in decomposition in streams dominated by shrub vegetation, but expect that decomposition rates may increase with increasing shading. If shrub vegetation increased its distribution, the amount of shading and the production of leaf-litter would increase (into the stream channel and the study area). The effect of increased shading may further reduce the already very low levels of primary production in the streams, and the macro invertebrate community may shift towards species that are specialized on feeding on leaf litter. Because of the structural properties of shrub vegetation, a moderate reduction of snow cover may not have as severe effects compared to streams draining areas with tundra and forest cover.

The novel part of my study included a focus on stream ecological functioning in tundra and shrub areas combined with the manipulation of snow depth. The study supports the importance of shading and vegetation cover on decomposition of leaf litter in open-canopy streams that drain catchments with shrub and tundra vegetation, which has also been suggested for streams at lower longitudes and altitudes (e.g. Leberfinger *et al.* (2011)). Further, I expected shrub to show an intermediate pattern in the measured processes. To some extent, that expectation has been confirmed by the present study, but manipulation of snow depth has also revealed a property of shrub vegetation that was not predicted, namely the relative lack of effects caused by reductions in snow depth reflecting the vegetation’s capacity to accumulate or retain snow that may buffer the microenvironment against temperature changes. The effect of snow depth manipulation remains unclear. However, the very limited decomposition at the manipulated tundra site compared to the control sites indicated that stream ecological functioning in tundra might be vulnerable to climate change.

My results could not show to what extent vegetation cover and a reduction of snow depth affected the functional integrity of boreal headwater streams. Specific measures could be applied to improve the present study design, i.e. more studies with a greater number of replicates, longer duration (more than one season e.g. Kreyling *et al.* (2012), and further development of the chosen methodology. However, the resources necessary to repeat this study at a greater scale or to do a more fundamental study should be taken into consideration. Additionally, headwater streams are vulnerable to naturally occurring events (e.g. variations in water flow), hence there will always be an aspect of risk of failure in every field study of this kind. This risk should be worth the research effort. Yet, the results of the present study may suggest a specific focus on open-canopy streams in areas with predominantly shrub and tundra vegetation. Hence, alternative and less extensive studies could be proposed. One such alternative could be a manipulation of the snow accumulating capacity in tundra and forest. Another possible study could be to manipulate the introduction of leaf litter into the stream to assess how the amount of leaf-litter is affecting functional traits such as decomposition rates. A third possibility could be a screening of potential food sources in open-canopy streams following the surprisingly limited periphyton growth found in the present study to better understand the isotopic variation found in the macroinvertebrate species. For, with the already ongoing climate change and alteration of vegetation cover, knowledge about stream ecological functioning may provide important information about ecosystem status and possible long term scenarios.

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Appendix

Appendix 1: Vegetation mapping classes

Table A1. Classes used for vegetation mapping.

Class	Class description
be	*Betula nana*
B	*Betula tortuosa*
sa	*Salix* spp.
ju	*Juniperus communis*
Woody	Deciduous and evergreen dwarf shrubs
nonWoody	Non woody plants
Wa	Water
mo	Mosses (including *Sphagnum* spp.)
nonVeg	Stone, bare ground, litter
li	Lichens

Appendix 2: Hemiphotos taken for the calculation of the Shade Proportion Index (SPI)

Figure A1. Illustration of a hemiphoto taken in the bottom layer at the shrub site KS8 (left) and the resulting binary B/W picture (right) that was used to calculate the Shade Proportion Index (SPI) by using the image tool ImageJ with the radial profile plugin.

Appendix 3: Water chemistry data

Table A2. Water chemistry data from autumn 2012 and spring 2013. No water samples were collected at site KT2 in autumn because the streambed was dry and at site KS3 in spring since the site was covered by snow. The variation in pH, alkalinity (mmol*l^{-1}) and carbon (mg C/l) was biologically insignificant. Downstream snow depth manipulated sites are shown in italics, upstream control sites are labeled by '-2' (e.g. KF6-2).

		Autumn			**Spring**	
Site	**pH**	**alkalinity**	**Carbon**	**pH**	**alkalinity**	**Carbon**
KF1	7.55	0.379	1.4	7.26	0.373	2.9
KF2	7.59	0.443	1.0	7.17	0.205	1.8
KF3	7.18	0.428	1.0	7.09	0.304	2.0
KF4	7.60	0.441	1.0	7.14	0.206	1.9
KF5	7.71	0.837	3.2	7.61	0.643	4.4
KF6	7.54	0.541	2.8	7.07	0.279	5.7
KF6-2	7.43	0.502	3.1	7.11	0.266	5.4
KS1	7.49	0.348	1.8	7.20	0.272	3.7
KS3	7.45	0.390	2.2	NA	NA	NA
KS4	7.30	0.357	2.1	7.03	0.254	4.1
KS5	7.79	0.843	1.5	7.62	0.641	3.2
KS5-2	7.39	0.396	2.7	7.62	0.633	3.0
KS8	7.72	0.905	2.7	7.62	0.669	4.2
KS9	7.85	1.047	1.6	7.45	0.495	4.4
KT1	7.66	0.441	0.8	7.17	0.200	1.6
KT2	NA	NA	NA	7.35	0.450	3.4
KT3	7.76	0.795	1.1	7.59	0.633	3.8
KT4	7.94	1.274	2.3	7.47	0.645	3.8
KT5	7.99	1.179	2.1	7.71	0.743	3.8
KT5-2	7.84	1.140	2.4	7.64	0.750	3.8
KT6	7.65	0.542	2.2	7.16	0.225	5.2

Appendix 4: Data logger temperatures

Table A3. Temperature data measured at soil depths 1cm and 10cm and at the stream bottom (Water). Min., minimum temperature; days < 0, number of days with subzero temperatures; mean < 0, mean temperature during the days with subzero temperature.

Site	**Soil depth 1 cm**			**Soil depth 10 cm**			**Water**		
	Min.	**days < 0**	**mean < 0**	**Min.**	**days < 0**	**mean < 0**	**Min.**	**days < 0**	**mean < 0**
KF1	-0,7	133	-0,4	-0,5	126	-0,3	-0,8	56	-0,2
KF2	-0,8	112	-0,4	-0,5	71	-0,2	-1,5	24	-0,4
KF3	-0,2	80	-0,1	-0,2	87	-0,1	0,2	0	NA
KF4	-0,7	135	-0,4	-0,4	84	-0,2	0,1	0	NA
KF5	-1,1	131	-0,5	-0,4	105	-0,2	0,1	0	NA
KF6	-13,9	150	-3,2	-10,6	148	-2,5	-3,5	122	-1,1
KF6-2	-0,9	138	-0,4	-0,5	137	-0,2	-0,1	2	-0,1
KS1	-1,8	133	-0,8	-1,3	131	-0,7	-0,2	18	-0,1
KS3	0,1	0	NA	0,1	0	NA	-0,6	10	-0,4
KS4	0,1	0	NA	0,1	0	NA	0,2	0	NA
KS5	-8,7	133	-2,3	-7,2	139	-1,8	0,2	0	NA
KS5-2	-0,5	137	-0,2	-0,1	40	-0,1	0,1	0	NA
KS8	-2,7	143	-0,9	-0,9	113	-0,4	0,1	0	NA
KS9	-1,9	136	-0,8	-1,0	115	-0,5	0,2	0	NA
KT1	-1,1	144	-0,5	-0,9	151	-0,4	-0,1	5	-0,1
KT2	1,2	0	NA	-2,0	148	-0,9	-0,3	38	-0,2
KT3	-0,2	81	-0,1	-0,1	19	-0,1	0,2	0	NA
KT4	-1,5	169	-0,6	-0,9	137	-0,4	0,0	3	0,0
KT5	-17,8	178	-4,5	-15,3	158	-4,3	-8,3	140	-2,7
KT5-2	-4,0	150	-1,8	-3,5	158	-1,4	0,4	0	NA
KT6	-4,7	171	1,5	-1,3	141	-0,6	0,2	0	NA

Appendix 5: Effect of snow depth manipulation on soil temperatures

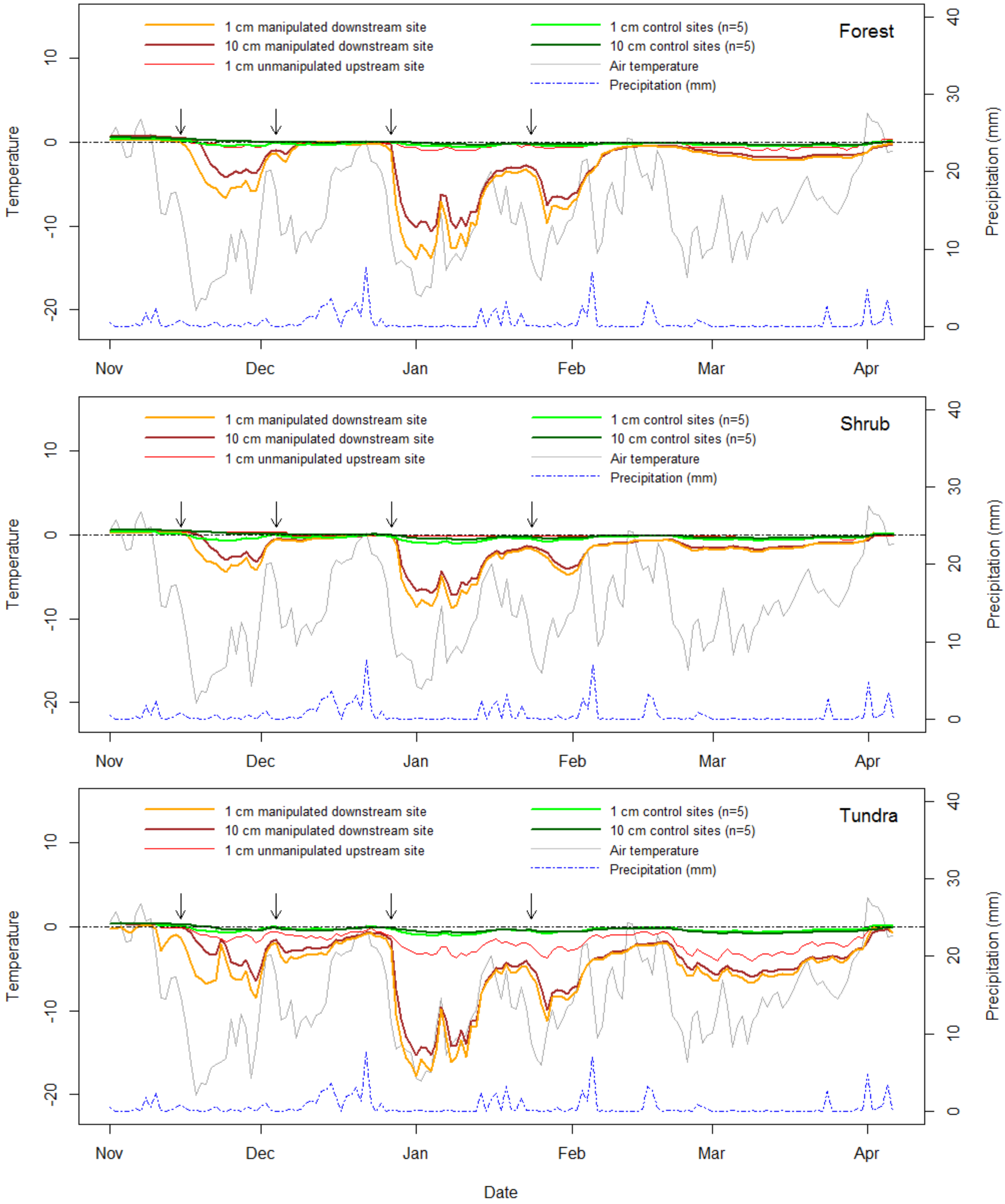

Figure A2. Effect of snow depth manipulation on soil temperatures (in °C) at a depth of 1 and 10 cm at manipulated forest, shrub and tundra sites. Arrows indicate the timing of the manipulation. Temperature data at 10 cm at upstream control sites of all land cover types were removed because of data logger errors. For further details, see Appendix 4.

Appendix 6: Shade proportion index by site

Table A4. Treatment and shading at the studied sites. Note: C, control; UC, upstream control, SDM, snow depth manipulation.

Site	Land cover	Treatment	SPI
KF1	Forest	C	6.241
KF2	Forest	C	7.655
KF3	Forest	C	7.452
KF4	Forest	C	7.344
KF5	Forest	C	8.969
KF6	Forest	SDM	6.609
KF6-2	Forest	UC	7.342
KS1	Shrub	C	5.023
KS3*	Shrub	C	NA
KS4	Shrub	C	5.942
KS5	Shrub	SDM	5.222
KS5-2	Shrub	UC	5.954
KS8	Shrub	C	5.921
KS9	Shrub	C	4.839
KT1	Tundra	C	5.134
KT2	Tundra	C	4.587
KT3	Tundra	C	5.410
KT4	Tundra	C	4.724
KT5	Tundra	SDM	3.757
KT5-2	Tundra	UC	4.957
KT6	Tundra	C	4.707

Appendix 7: Pairwise comparisons of isotopic values (two-sample K–S tests)

Table A5. Pairwise comparisons of C isotopic values of potential food sources at control sites, upstream control sites and manipulated downstream sites. With D as the greatest distance between distributions.

Isotope	Treatment	Season	Species	N	Mean	± SD	Betula det (D, p)	Salix (D, p)	Salix det (D, p)	Bryophytes (D, p)
C	C	Spring	Betula	12	-31.97	0.83	0.25, 0.869	0.75, 0.001	0.67, 0.008	0.56, 0.016
C	C	Spring	Betula det	12	-31.82	0.65		0.83, < 0.001	0.67, 0.008	0.56, 0.016
C	C	Spring	Salix	12	-29.76	1.25			0.50, 0.100	0.56, 0.016
C	C	Spring	Salix det	12	-30.50	0.77				0.56, 0.016
C	C	Spring	Bryophytes	18	-33.50	5.47				
C	C	Autumn	Betula	12	-31.66	0.58	0.17, 0.999	0.83, < 0.001	0.95, < 0.001	0.72, < 0.001
C	C	Autumn	Betula det	12	-31.78	0.74		0.75, < 0.001	0.86, < 0.001	0.64, 0.003
C	C	Autumn	Salix	18	-29.68	1.32			0.23, 0.605	0.72, < 0.001
C	C	Autumn	Salix det	19	-29.82	0.71				0.83, < 0.001
C	C	Autumn	Bryophytes	18	-34.44	4.56				
C	UC	Spring	Betula	12	-31.44	1.17	0.25, 0.869	0.75, 0.001	0.67, 0.008	0.39, 0.192
C	UC	Spring	Betula det	12	-31.08	1.48		0.75, 0.001	0.67, 0.008	0.39, 0.192
C	UC	Spring	Salix	12	-28.76	1.49			0.42, 0.256	0.47, 0.063
C	UC	Spring	Salix det	12	-29.63	1.39				0.39, 0.192
C	UC	Spring	Bryophytes	18	-32.64	5.24				
C	UC	Autumn	Betula	12	-30.85	1.01	0.42, 0.256	0.44, 0.095	0.31, 0.454	0.78, < 0.001
C	UC	Autumn	Betula det	12	-31.47	0.50		0.67, 0.002	0.56, 0.016	0.78, < 0.001
C	UC	Autumn	Salix	18	-30.09	1.51			0.33, 0.275	0.72, < 0.001
C	UC	Autumn	Salix det	18	-30.30	1.57				0.78, < 0.001
C	UC	Autumn	Bryophytes	18	-36.41	5.04				
C	SDM	Spring	Betula	11	-31.59	0.66	0.40, 0.225	0.78, < 0.001	0.67, 0.002	0.61, 0.007
C	SDM	Spring	Betula det	12	-31.72	1.09		0.69, < 0.001	0.53, 0.026	0.61, 0.006
C	SDM	Spring	Salix	18	-29.47	1.35			0.22, 0.781	0.61, 0.002
C	SDM	Spring	Salix det	18	-29.76	1.70				0.61, 0.002
C	SDM	Spring	Bryophytes	18	-34.83	5.71				
C	SDM	Autumn	Betula	12	-31.53	0.62	0.45, 0.108	0.67, 0.002	0.81, < 0.001	0.71, < 0.001
C	SDM	Autumn	Betula det	13	-31.06	0.59		0.59, 0.006	0.75, < 0.001	0.71, < 0.001
C	SDM	Autumn	Salix	18	-29.99	1.28			0.36, 0.167	0.71, < 0.001
C	SDM	Autumn	Salix det	16	-29.26	1.34				0.76, < 0.001
C	SDM	Autumn	Bryophytes	17	-35.79	4.66				

Table A6. Pairwise comparisons of N isotopic values of potential food sources at control sites, upstream control sites and manipulated downstream sites. With D as the greatest distance between distributions.

Isotope	Treatment	Season	Species	N	Mean	± SD	Betula det (D, p)	Salix (D, p)	Salix det (D, p)	Bryophytes (D, p)
N	C	Spring	Betula	12	-1.42	1.17	0.17, 0.999	0.42, 0.256	0.25, 0.869	0.69, < 0.001
N	C	Spring	Betula det	12	-1.41	1.01		0.50, 0.100	0.33, 0.536	0.67, 0.002
N	C	Spring	Salix	12	-0.24	1.41			0.42, 0.256	0.50, 0.042
N	C	Spring	Salix det	12	-1.13	1.18				0.69, < 0.001
N	C	Spring	Bryophytes	18	1.16	1.52				
N	C	Autumn	Betula	12	0.38	1.94	0.25, 0.869	0.42, 0.136	0.39, 0.148	0.58, 0.010
N	C	Autumn	Betula det	12	-0.03	1.63		0.28, 0.581	0.32, 0.348	0.67, 0.002
N	C	Autumn	Salix	18	0.26	0.84			0.16, 0.935	0.56, 0.007
N	C	Autumn	Salix det	19	0.36	1.02				0.57, 0.002
N	C	Autumn	Bryophytes	18	1.48	1.10				
N	UC	Spring	Betula	12	-1.74	0.52	0.25, 0.869	0.75, 0.001	0.58, 0.031	0.94, < 0.001
N	UC	Spring	Betula det	12	-1.82	0.77		0.58, 0.034	0.42, 0.256	0.94, < 0.001
N	UC	Spring	Salix	12	-0.67	1.02			0.25, 0.869	0.56, 0.016
N	UC	Spring	Salix det	12	-0.97	0.86				0.72, < 0.001
N	UC	Spring	Bryophytes	18	1.50	2.07				
N	UC	Autumn	Betula	12	-1.22	0.73	0.33, 0.536	0.64, 0.003	0.47, 0.063	0.94, < 0.001
N	UC	Autumn	Betula det	12	-1.04	0.94		0.42, 0.136	0.36, 0.262	0.89, < 0.001
N	UC	Autumn	Salix	18	-0.17	0.98			0.22, 0.781	0.56, 0.007
N	UC	Autumn	Salix det	18	-0.70	1.39				0.67, < 0.001
N	UC	Autumn	Bryophytes	18	1.07	0.93				
N	SDM	Spring	Betula	12	-0.97	1.35	0.42, 0.256	0.25, 0.706	0.25, 0.706	0.72, < 0.001
N	SDM	Spring	Betula det	12	-1.08	0.88		0.33, 0.355	0.28, 0.581	0.83, < 0.001
N	SDM	Spring	Salix	18	-0.92	0.90			0.22, 0.781	0.83, < 0.001
N	SDM	Spring	Salix det	18	-0.70	1.43				0.78, < 0.001
N	SDM	Spring	Bryophytes	18	1.44	1.53				
N	SDM	Autumn	Betula	12	-0.88	1.15	0.30, 0.518	0.28, 0.581	0.15, 0.994	0.86, < 0.001
N	SDM	Autumn	Betula det	13	-0.95	1.48		0.37, 0.188	0.29, 0.485	0.71, < 0.001
N	SDM	Autumn	Salix	18	-0.38	1.21			0.35, 0.196	0.67, < 0.001
N	SDM	Autumn	Salix det	16	-1.15	1.05				0.94, < 0.001
N	SDM	Autumn	Bryophytes	18	1.56	0.99				

Table A7. Pairwise comparisons of C isotopic values of macroinvertebrate species at control sites, upstream control sites and manipulated downstream sites. With D as the greatest distance between distributions.

Isotope	Treatment	Season	Species	N	Mean	± SD	N (D, p)	B (D, p)	L (D, p)	A (D, p)
C	C	Spring	C	2	-28.31	0.78	1.00, 0.026	1.00, 0.056	0.50, 0.750	NA
C	C	Spring	N	11	-32.33	3.69		0.64, 0.036	1.00, < 0.001	NA
C	C	Spring	B	7	-33.82	0.74			1.00, < 0.001	NA
C	C	Spring	L	7	-27.77	0.23				NA
C	C	Spring	A	0	NA	NA				
C	C	Autumn	C	18	-30.51	0.70	0.61, 0.017	1.00, < 0.001	0.78, 0.004	1.00, 0.105
C	C	Autumn	N	8	-31.68	0.93		1.00, 0.002	1.00, < 0.001	1.00, 0.222
C	C	Autumn	B	5	-35.73	0.39			1.00, 0.004	1.00, 0.333
C	C	Autumn	L	6	-28.63	1.07				1.00, 0.286
C	C	Autumn	A	1	-37.82	NA				
C	UC	Spring	C	22	-30.24	1.62	0.34, 0.151	0.62, 0.001	0.95, < 0.001	NA
C	UC	Spring	N	18	-29.80	1.34		0.89, < 0.001	1.00, < 0.001	NA
C	UC	Spring	B	15	-32.76	1.18			1.00, < 0.001	NA
C	UC	Spring	L	8	-27.34	0.49				NA
C	UC	Spring	A	0	NA	NA				
C	UC	Autumn	C	23	-29.60	1.78	0.69, < 0.001	0.96, < 0.001	0.36, 0.181	0.65, 0.004
C	UC	Autumn	N	21	-31.64	1.07		1.00, < 0.001	0.88, < 0.001	0.46, 0.105
C	UC	Autumn	B	14	-34.72	0.48			1.00, < 0.001	1.00, < 0.001
C	UC	Autumn	L	13	-28.82	0.89				0.92, < 0.001
C	UC	Autumn	A	9	-31.19	0.64				
C	SDM	Spring	C	3	-28.92	0.86	0.69, 0.116	1.00, 0.009	0.86, 0.067	NA
C	SDM	Spring	N	16	-30.49	1.96		0.81, < 0.001	1.00, < 0.001	NA
C	SDM	Spring	B	9	-32.78	1.01			1.00, < 0.001	NA
C	SDM	Spring	L	7	-26.98	0.52				NA
C	SDM	Spring	A	0	NA	NA				
C	SDM	Autumn	C	15	-29.16	1.45	1.00, 0.002	1.00, < 0.001	0.60, 0.033	NA
C	SDM	Autumn	N	3	-33.49	0.82		0.91, 0.022	1.00, 0.012	NA
C	SDM	Autumn	B	11	-34.75	0.93			1.00, < 0.001	NA
C	SDM	Autumn	L	8	-28.54	0.19				NA
C	SDM	Autumn	A	0	NA	NA				

Table A8. Pairwise comparisons of N isotopic values of macroinvertebrate species at control sites, upstream control sites and manipulated downstream sites. With D as the greatest distance between distributions.

Isotope	Treatment	Season	Species	N	Mean	± SD	N (D, p)	B (D, p)	L (D, p)	A (D, p)
N	C	Spring	C	2	0.64	0.29	1.00, 0.026	1.00, 0.056	1.00, 0.056	NA
N	C	Spring	N	11	1.66	0.46		0.77, 0.006	1.00, < 0.001	NA
N	C	Spring	B	7	2.55	0.38			1.00, < 0.001	NA
N	C	Spring	L	7	3.40	0.24				NA
N	C	Spring	A	0	NA	NA				
N	C	Autumn	C	18	0.65	0.89	0.33, 0.477	1.00, < 0.001	0.83, 0.002	0.94, 0.211
N	C	Autumn	N	8	0.97	0.61		1.00, 0.002	0.71, 0.043	0.88, 0.444
N	C	Autumn	B	5	2.79	0.17			0.33, 0.818	1.00, 0.333
N	C	Autumn	L	6	2.39	0.89				0.83, 0.571
N	C	Autumn	A	1	NA	NA				
N	UC	Spring	C	22	-0.11	0.89	0.81, < 0.001	1.00, < 0.001	1.00, < 0.001	NA
N	UC	Spring	N	18	1.16	0.38		0.83, < 0.001	1.00, < 0.001	NA
N	UC	Spring	B	15	2.18	0.42			0.87, < 0.001	NA
N	UC	Spring	L	8	3.20	0.36				NA
N	UC	Spring	A	0	NA	NA				
N	UC	Autumn	C	23	-0.17	0.65	0.78, < 0.001	0.96, < 0.001	0.96, < 0.001	0.54, 0.028
N	UC	Autumn	N	21	1.15	0.49		0.71, < 0.001	0.92, < 0.001	0.67, 0.003
N	UC	Autumn	B	14	2.36	0.72			0.34, 0.343	0.82, < 0.001
N	UC	Autumn	L	13	2.74	0.65				0.92, < 0.001
N	UC	Autumn	A	9	0.41	0.59				
N	SDM	Spring	C	3	0.48	0.72	0.81, 0.041	0.89, 0.036	1.00, 0.017	NA
N	SDM	Spring	N	16	1.39	0.27		0.83, < 0.001	1.00, < 0.001	NA
N	SDM	Spring	B	9	1.88	0.63			0.89, 0.001	NA
N	SDM	Spring	L	7	3.15	0.34				NA
N	SDM	Spring	A	0	NA	NA				
N	SDM	Autumn	C	15	0.59	1.05	0.47, 0.571	0.91, < 0.001	1.00, < 0.001	NA
N	SDM	Autumn	N	3	1.22	0.36		0.91, 0.022	1.00, 0.012	NA
N	SDM	Autumn	B	11	2.50	0.55			1.00, < 0.001	NA
N	SDM	Autumn	L	8	3.32	0.25				NA
N	SDM	Autumn	A	0	NA	NA				

Appendix 8: Isotopic bi-plots with bryophytes.

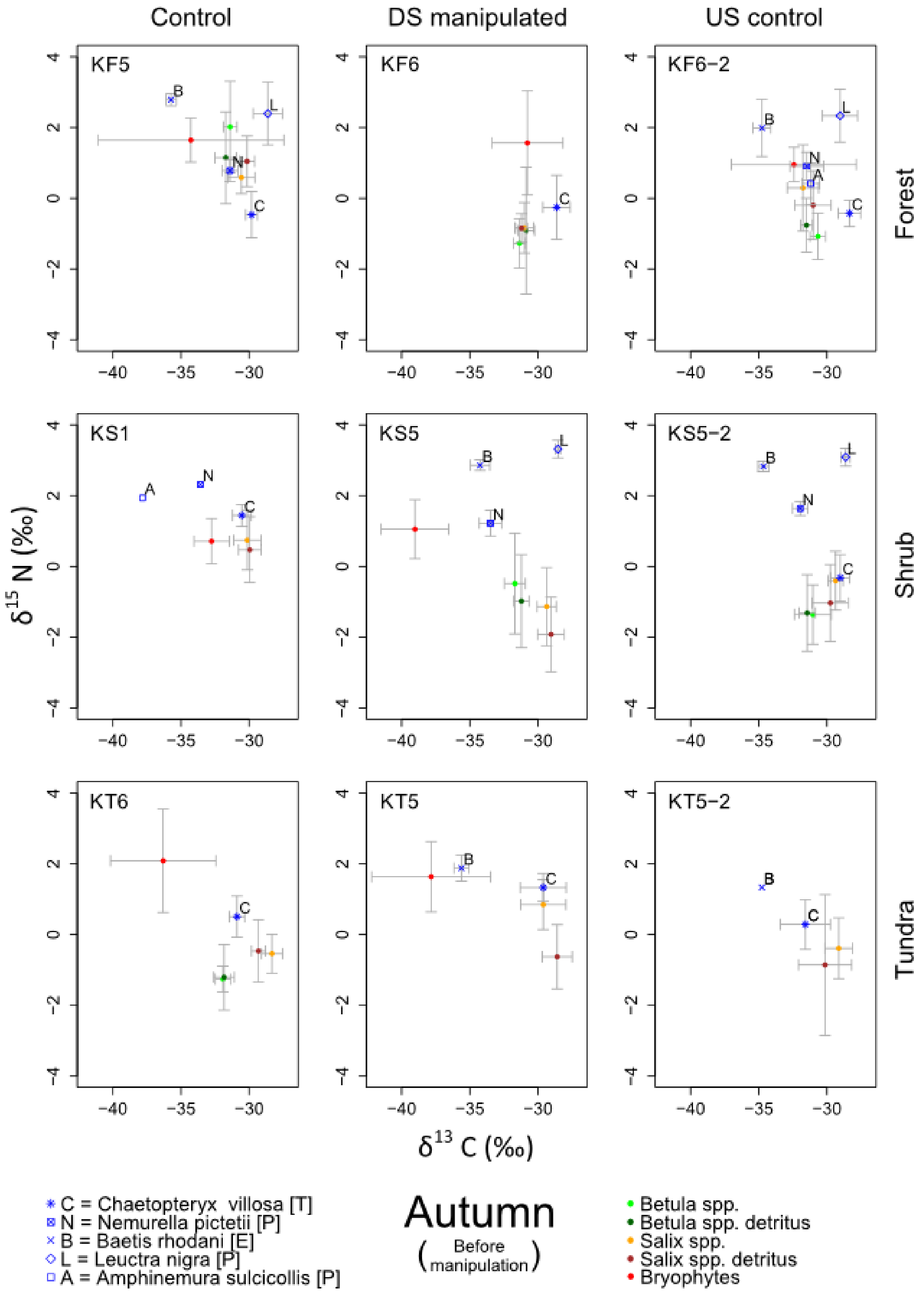

Figure A3. Isotopic bi-plots of mean δ^{13}C and mean δ^{15}N values of potential food sources and macro invertebrate species at nine sites in the autumn (prior to manipulation) including bryophyte values.

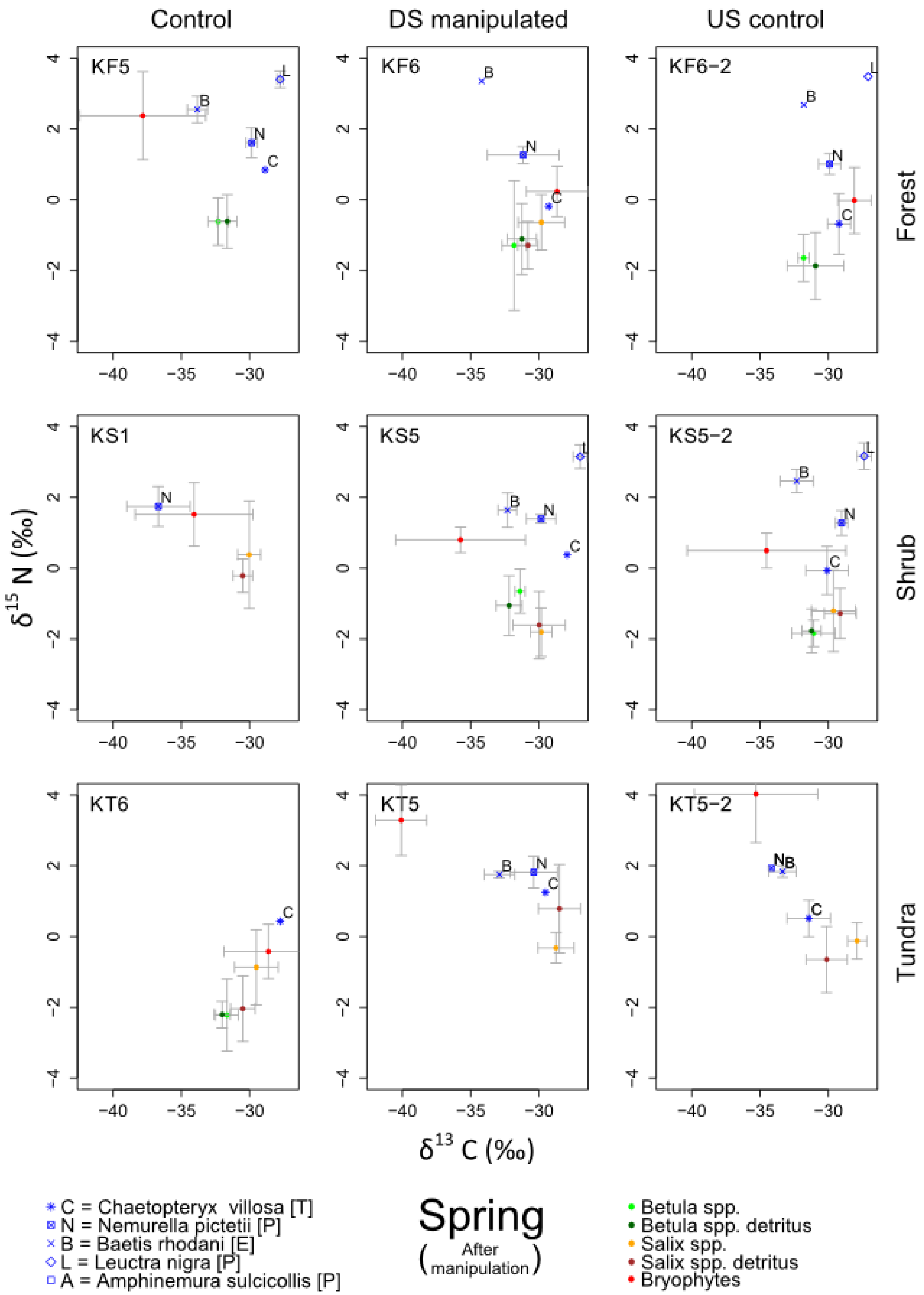

Figure A4. Isotopic bi-plots of mean δ^{13}C and mean δ^{15}N values of potential food sources and macro invertebrate species at nine sites in the spring (following manipulation) including bryophyte values.