Rhizosphere allocation by canopy-forming species dominates soil CO_2 efflux in a subarctic landscape

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Abstract:

- In arctic ecosystems, climate change has increased plant productivity. As arctic carbon (C) stocks are predominantly located below ground, the effects of greater plant productivity on soil C storage will significantly determine the net sink/source potential of these ecosystems, but vegetation controls on soil CO_2 efflux remain poorly resolved.

- To identify the role of canopy forming species in below-ground C dynamics, we conducted a girdling experiment with plots distributed across 1 km^2 of treeline birch (*Betula pubescens*) forest and willow (*Salix lapponum*) patches in northern Sweden and quantified the contribution of canopy vegetation to soil CO_2 fluxes and below-ground productivity.
- Girdling birches reduced total soil CO_2 efflux in the peak growing season by 53% - double the expected amount given that trees contribute only half of the total leaf area in the forest. Root and mycorrhizal mycelial production also decreased substantially. At peak season, willow shrubs contributed 38% to soil CO_2 efflux in their patches.
- Our findings indicate that C, recently fixed by trees and tall shrubs, makes a substantial contribution to soil respiration. It is critically important that these processes are taken into consideration in the context of a greening arctic since productivity and ecosystem C sequestration are not synonymous.

Introduction

Climate warming is causing large-scale increases in primary productivity in much of the terrestrial Arctic (Myers-Smith *et al.*, 2020), as predicted by long-term warming experiments (Elmendorf *et al.*, 2012a) and vegetation models (Yu *et al.*, 2017). Where changes in ecosystem productivity are occurring, they are driven by increased growth of tundra vegetation (Elmendorf *et al.*, 2012b; Bjorkman *et al.*, 2018), but also often by an increase in cover and geographical range of deciduous shrub species (Myers-Smith *et al.*, 2011). Above-ground carbon (C) accumulation at northern high latitudes, following increased productivity, is projected to continue into the next century (Qian *et al.*, 2010). There is also clear evidence, from responses of trees to historical changes in climate, and global gradient studies, that arctic and alpine treelines are influenced by climate and that forests will expand if climate continues to warm (Richardson & Friedland, 2009). Poleward and altitudinal shifts of treelines have already been observed in some locations (Wilmking *et al.*, 2006; Harsch *et al.*, 2009; Hofgaard *et al.*, 2013; Hagedorn *et al.*, 2014), although responses are heterogeneous due both to historical and on-going land use and grazing pressure. Forest expansion in the near future will only influence the tundra close to the present treeline, but significant increases in productivity have also been observed in large parts of the low arctic tundra (Reichle *et al.*, 2018). These subzones are found where deciduous shrub species are present, and often dominant, in the plant community (Walker *et al.*, 2005). Shrubs in the tundra grow taller and expand their spatial range in response to a warmer climate (Myers-Smith *et al.*, 2011, 2019a), and are the most likely plant group to increase in dominance across large areas of the low Arctic in this and the next century (Pearson *et al.*, 2013).

Above-ground biomass in the most productive tundra subzones has increased by up to 0.1 kg C m^{-2} to approximately 0.5 kg m^{-2} between 1982 and 2010 (Epstein *et al.*, 2012). However, this stock of biomass C is small compared to soil C stocks. Tundra soils in the majority of the treeless Arctic store up to 50 kg C m^{-2} and the highest densities of C are commonly found in the top 30 cm of the profile (Kuhry *et al.*, 2013; Siewert, 2018), along with almost all of plant root biomass (Jackson *et al.*, 1996; Iversen *et al.*, 2015). This stock surpasses by far the aboveground C storage even in fully forested boreal (Siewert *et al.*, 2015) and subarctic forests (Hartley *et al.*, 2012). Increasing photosynthetic biomass in the

Arctic results in more C entering the ecosystem, and there is much interest in the ecosystem feedbacks that may result (Myers-Smith *et al.*, 2011, 2019b). However, primary productivity is just one facet of the terrestrial C cycle, and the fate of assimilated C must also be understood, on timescales varying from minutes to millennia, to enable a forecasting of future ecosystem C storage.

The task of linking above-ground changes in GPP to total ecosystem storage of C is complex. Most C fixed by arctic vegetation is allocated below-ground (Street *et al.*, 2018), where the majority of plant biomass is located (Iversen *et al.*, 2015). GPP can be robustly characterised in tundra based on leaf area and basic meteorological data (Shaver *et al.*, 2007), meaning that GPP may be predicted by changes in above-ground canopy properties that can be detected via remote sensing (Epstein *et al.*, 2012). However, the change in ecosystem respiration with increasing shrub and tree encroachment is much more challenging to predict. For example, tall deciduous shrub species that are structurally similar aboveground (*Betula* and *Alnus*) allocate C belowground very differently in relation to nitrogen acquisition (Street *et al.*, 2018), which may, in turn, affect C turnover rates in the soil. The fate of photosynthesised C within an ecosystem may therefore differ significantly between contrasting arctic plant communities.

Soil CO_2 efflux constitutes the largest component of ecosystem C losses; in many forest systems soil CO_2 efflux comprises, in roughly equal measure, of heterotrophic and autotrophic sources (Bond-Lamberty *et al.*, 2004; Subke *et al.*, 2006). The ratios of heterotrophic to autotrophic contributions to the total soil CO_2 efflux are less well characterised in tundra (Shaver *et al.*, 2007; Hicks Pries *et al.*, 2015), but this information is required in order to understand C budgets. As tall shrubs and trees represent future plant communities, given further climate change (Pearson *et al.*, 2013), it is particularly important to quantify and understand their contribution to soil CO_2 efflux within their present distribution. Quantifying the contribution of recent plant C inputs to soil CO_2 efflux is technically challenging, usually requiring either destructive methods or isotopic labelling techniques to partition autotrophic and heterotrophic CO_2 sources (Subke *et al.*, 2006). Previous trenching and clipping approaches in these ecosystems have caused considerable disturbance, altered soil thermal and moisture regimes, and have generally only been able to quantify the total contributions of all vegetation, including short-stature species, to

ecosystem fluxes (Hartley *et al.*, 2012). Stem girdling halts the delivery of photosynthate from canopies to below ground by disrupting the phloem tissue while limiting the reduction in movement of water to the rest of the plant through the xylem, while leaves remain alive. This method therefore makes it possible to identify the contribution of canopy-forming species to soil CO_2 efflux, even where extensive understorey plant communities remain, and provides a unique insight into the role of canopy species and associated ectomycorrhizal (ECM) fungi in controlling C fluxes from the soil (Högberg *et al.*, 2001).

Alongside plant root respiration, respiration from extraradical ECM mycelium can contribute 15-25 % of the total soil CO_2 efflux in boreal and temperate forests (Heinemeyer *et al.*, 2007; Hasselquist *et al.*, 2012; Hagenbo *et al.*, 2019) and ECM mycelial necromass has been linked with fast decomposition (Drigo *et al.*, 2012; Clemmensen *et al.*, 2015). Furthermore, low stocks of soil C in treeline forests (compared to adjacent tundra) may be linked to enzymatic oxidation of organic matter by ECM fungi, as they extract organic forms of N (Bödeker *et al.*, 2014), and to a broader rhizosphere priming effect by birch trees and their symbionts (Hartley *et al.*, 2012). In such a system, where canopy-assimilated C is in high demand for the acquisition of N and other nutrients by symbiotic fungi, a large proportion of soil CO_2 efflux should be linked to the C supply from the canopy. In contrast, tundra willow shrub communities typically grow in riparian zones and in areas of deep snow cover, where soil moisture and mineral nutrient influx is higher than in other tundra types (Nadelhoffer *et al.*, 1991; Sturm *et al.*, 2005), potentially reducing plant investment in ECM fungi (Treseder, 2004). Furthermore, high soil moisture and occasional anoxia are not favourable to many ECM fungi, and can limit their growth within the soil matrix (Lodge, 1989; Wurzburger *et al.*, 2004; Barnes *et al.*, 2018), thus reducing the demand for assimilated C. Willow shrubs have been widely documented to increase in growth and cover in response to climate change (Tape *et al.*, 2006; Forbes *et al.*, 2010; Myers-Smith *et al.*, 2019a), therefore it is important to understand C cycling in this ecosystem at present, in order to predict changes in the future.

Flux partitioning experiments have seldom been done in arctic ecosystems (Subke *et al.*, 2006), and the relative influence of the canopy has never been elucidated by girdling. The problem of partitioning is exacerbated by the diversity and heterogeneity of tundra and treeline plant communities with contrasting dominant plant species (Walker *et al.*, 2005).

Quantitative information on rhizosphere processes in contrasting treeline and tundra plant communities in relation to plant productivity is essential to underpin a better understanding of variations in landscape soil CO_2 efflux. To address these issues, we conducted a girdling experiment at plots across a sub-arctic landscape in northern Sweden to isolate and test the importance of canopy inputs for soil CO_2 efflux and below-ground productivity.

Past experiments that partitioned autotrophic and heterotrophic CO_2 fluxes in boreal and northern temperate forests, using stem girdling and trenching, were in situations where the canopy comprised the majority of leaf area (Högberg *et al.*, 2001; Subke *et al.*, 2006). In a subarctic birch forest, leaf area is likely more equally distributed between canopy and understorey vegetation. In this forest, trenching canopy roots and clipping the understorey reduced soil CO_2 efflux by 50 % in peak season (Hartley *et al.*, 2012). We therefore hypothesised (1) that the contribution by canopy dominant trees to autotrophic soil CO_2 effluxes would broadly reflect their contribution to the total leaf area of the community. Furthermore, we hypothesised (2) that autotrophic contribution to soil CO_2 efflux would be lower under tundra willow than under treeline forest alongside a lower investment in mycorrhizal fungi.

Methods

Site selection and experimental design

The experiment was located around a forest-tundra ecotone 3-4 km south of the Abisko Scientific Research Station, Sweden (68°18 N 18°49 E, ~600 m asl). The girdling experiment was carried out in mountain birch forest (*Betula pubescens* Ehrh. ssp *czerepanovii* (Orlova) Hämet Ahti) and willow thickets (Identified as *Salix lapponum* L. but there is very high potential for hybridisation in this genus (Forrest, 2006)) that were distributed across a 0.88 km^2 area (Fig. 1). The birch forests grow on well-drained spodosols, underlain by glacial till without permafrost (Sjögersten & Wookey, 2002). The understorey primarily comprises of ericaceous dwarf shrubs (*Empetrum nigrum* L. ssp *hermaphroditum* (Hagerup) Böcher*, Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L. and *Vaccinium uliginosum* L.) and feather mosses (e.g. *Hylocomium splendens* and *Pleurozium schreberi*) (Fig.S1a). Willow thickets (Fig.S1b) in this area typically grow in poorly drained, late snow-lie communities, alongside *Betula nana* L., with an herbaceous and graminoid understorey.

Prior to girdling, 5 willow and 6 mountain birch plots were established in early June 2017, with each plot divided into paired sub-plots. Pairs were selected to have similar tree and stem density, soil C stocks, soil C:N ratio (Table 1) and understorey (birch plots: ericaceous dwarf shrubs and mosses; willow plots: forbs and mosses). The birch sub-plots had a circular area with a radius of 10 m and an average tree density of 586 trees ha^{-1} (Table 1). The willow sub-plots had a radius of 2 m and a density of 5-6 stems m^{-2}, representing the largest plots with willow-only canopies that could be found in the study area. The larger size of the birch plots was necessary to ensure that all trees were girdled that could potentially be contributing to below-ground respiration at the central measurement area. The outer perimeters of paired birch sub-plots were separated by 10 – 20 m and the paired plots were separated from other pairs by between 300 to 1100 m (Fig 1a). For the willow plots, the distances between paired sub-plot outer perimeters were 2 - 16 m and pairs were separated by between 300 and 900 m. Each birch plot contained 3-4 trees within the central 3 m radius, within which all subsequent measurements were taken. Unlike in the relatively sparse birch forest, the lack of gaps between willow shrubs meant that it was also necessary to trench the perimeter of each willow plot, and plastic sheet was then inserted through the entire soil depth until rocks were encountered (top 10 – 30 cm of soil) to prevent roots from

adjacent plants from entering. Trenching was not carried out around the birch plots, because the size of the buffering area around the central 3 m radius was deemed sufficient to minimise edge effects.

Soil CO_2 efflux measurements (see Soil CO_2 Efflux section for methods) were carried out twice at all birch and willow plots after snow-melt (9-12 June 2017) but prior to the application of girdling treatments. Paired T-tests were carried out to test for a pre-girdling difference in plot characteristics between sub-plots and no significant differences were observed (Table 1). One sub-plot of each pair was girdled between 12th June 2017 and 15th June 2017. All birch stems over 1 cm in diameter were girdled within the 10 m radius plot. In the birch plots, a 4-8 cm section of the bark was removed around the circumference of each stem down to the xylem approximately 30 cm from the ground, leaving no phloem connection between leaves and roots (Högberg *et al.*, 2001). In the willow plots, every willow stem was girdled approximately 10-20 cm above the ground. Re-sprouting shoots from below the girdle-line were removed by hand whenever observed during the experiment. Birch and willow plants retained leaves until natural senescence in 2017 and all birch trees produced full leaves above the girdle-line in spring 2018. However, girdled willow canopies failed to produce leaves in 2018.

Soil CO_2 Efflux

Two days prior to the first efflux measurement and 5 - 7 days prior to girdling, three 5 cm tall, 15 cm inner-diameter, PVC collars were secured within 1 m of one of the three central trees of each birch plot. Collars were placed between under-storey stems and areas of moss mats in order to exclude live above-ground plant material. The collars were pushed firmly onto the soil and secured to the ground with non-setting plumber's putty (Evo-Stik Plumber's Mait®), to provide a good seal between collar and soil surface without severing shallow roots. The same method was applied to willow plots, with three collars placed within the central 1 m radius of the plot. Effectiveness of the collar seal using plumber's putty has been demonstrated by a linear increase in CO_2 concentrations when a closed chamber is attached to the collar (Parker *et al.*, 2015).

After girdling, CO_2 efflux was measured on 10 dates in the birch plots and 9 dates in the willow plots during the 2017 growing season, and 10 times each during the 2018 growing season. An EGM-5 infrared gas analyser (PP Systems International, Amesbury, MA, USA), with an attached CPY-5 darkened chamber, was used to measure soil CO_2 efflux (root-associated and heterotrophic activity). CO_2 efflux was calculated based on the linear increase in CO_2 concentration over 90 seconds. For each measurement date, all plots were visited on the same day between the hours of 09:00 and 18:00. The order in which plots (species and girdling treatment) and collars were sampled was alternated at every sampling day in order to minimise temporal sampling bias. The average soil CO_2 efflux value from the three collars was recorded as the true replicate flux per plot.

Soil and vegetation characteristics

To understand the effects of girdling on soil CO_2 efflux we measured plant and soil characteristics in the different plots. Soil organic C stocks of the organic horizon at each plot were calculated from a mean of nine soil cores (3.8 cm diameter) taken evenly across a 2 × 2 m area in the centre of the plot. The organic horizons from each core were separated from the lower mineral horizons, mixed together, oven-dried (60 °C) and weighed. C and nitrogen contents were measured on the combined sample in a Flash Smart™ elemental analyser (ThermoFisher Scientific, Waltham, MA, USA). Canopy leaf area index (LAI) was measured using an ACCUPAR LP-80 leaf area meter (Pullman, WA, USA) in early August in 2017 (all plots) and 2018 (birch plots only). At the birch plots, an average of 20 measurements taken evenly at 30 cm height across the north-south diameter of the plot was used. In the willow plots LAI was measured at 30 cm height at five points across the plot. The understorey LAI of each birch forest plot was estimated from the average NDVI of the visible forest floor in the 10 m radius of the plot from a drone platform according to relationships from a previous remote sensing study at 3 m scale at a nearby forest-tundra ecotone site (LAI = 0.00059 e9.502 NDVI (R^2 = 0.90) (Williams *et al.*, 2008)).

Drone imagery was taken on the 2nd August 2017 and 30th July 2018 in two flights using a senseFly eBee mapping drone (senseFly Inc., Switzerland) carrying a Parrot Sequoia multi-spectral sensor that delivers imagery in four spectral bands (Green, Red, Red Edge,

Near Infrared) and a separate RGB orthophoto. The drone was operated at a target elevation of 106 m resulting in an effective ground resolution of 10.3 cm (2017) and 11.2 cm (2018) in the final processed raster data of each flight. We used the Pix4Dmapper photogrammetric software (version 4.2.15, Pix4D, Lausanne, Switzerland) to combine individual images into continuous raster maps. We extracted an orthophoto and the normalized difference vegetation index (NDVI), which is considered an indicator of vegetation abundance and health (Rouse *et al.*, 1974). Each plot was visually marked in the field and later identified and outlined in the orthophoto composite using 4 m and 20 m diameter circles for willow and birch plots, respectively. The orthophoto was used to digitise manually the outline of the canopy of each individual tree in the birch plots and of the willow shrub coverage in willow plots. For birch plots, we extracted NDVI pixel values for the most centrally located trees in each plot. Understorey NDVI pixel values per plot were extracted from within each circle after masking out all tree canopies.

With every soil CO_2 efflux measurement from 24th July 2017 onwards, conductivity of the top 5 cm of soil was measured at all plots using a handheld HH2 ThetaProbe soil moisture meter (Delta-T Devices, Cambridge, UK). In birch plots, measurements were taken every meter in a 9 m^2 central square grid (16 measurements), and in the willow plots nine measurements were taken in a 4 m^2 square grid. Soil temperature at 5 cm depth was measured three times across the grid using a hand-held digital thermometer. Average temperature and conductivity values were calculated for each plot on each sampling date, then conductivity was converted to gravimetric moisture content according to:

$$\text{Gravimetric moisture (\%)} = e^{(a+Mb)}$$

Where M is the soil conductivity measured in the field and a and b are estimated based on the fitted relationship between gravimetric moisture and soil conductivity measured during a dry-down curve of saturated ericaceous peat from near the study plots ($a = 4.402$, $b = 0.00129$, Adjusted $R^2 = 0.955$). Using the bulk density of the calibration soil, gravimetric moisture was converted to volumetric moisture. To capture continuous volumetric soil moisture and soil temperature dynamics through timespan of the experiment, EC 5 soil moisture and TMB temperature smart sensors (Onset, Bourne, MA, USA) were installed at 5 cm depth in one birch and one willow plot. The probes logged hourly measurements to HOBO microstation loggers (Onset, Bourne, MA, USA).

Growing season root production and birch copy numbers

Root production over the growing season was estimated using ingrowth bags (Sullivan *et al.*, 2007). Cylindroid fibre-glass mesh bags (6 cm deep and 2.5 x 1.5 cm wide with a mesh size of 2 mm) were loosely packed with ericaceous peat. The peat was collected within the study landscape, dried for 48 h at 85°C, and sieved through a 4 mm mesh, with remaining roots picked out by hand prior to deployment in the bags. Ensuring maximum contact with the native soil, a root ingrowth bag was inserted vertically into the top six cm of organic soil, below the litter and moss layers, 30 cm from every CO_2 efflux collar in the willow and birch plots. Bags remained in the soil from 14th June 2017 until 18th September 2017, and new bags were inserted from 2nd June 2018 until 12th September 2018 with a total of 96 and 102 days field incubation per respective growing season. Bags were retrieved from the soil by carefully running a scalpel around each bag to a depth of 6 cm. Outside portions of in-grown roots were cut off in the lab and all roots inside the core were extracted, washed and dried at 60 °C for 72 hours, after which dry mass was recorded. C content of the roots was then analysed using a Flash Smart^{TM} elemental analyser.

For a species-specific assay, subsamples (0.7 - 30 mg (depending on amounts remaining after other analyses)) of dried in-growth roots from birch plots were finely milled by steel nuts (40 s at 5000 rpm) in 2 ml tubes (Precellys, Bertin Instruments, Germany), and DNA was extracted using the NucleoSpin soil kit (Macherey-Nagel, Düren, Germany). Copy numbers of the ITS region of *Betula* sp. were analysed by quantitative PCR (qPCR) using birch-specific primers (ITSb_F and ITSb_R) and a Biorad iQ5 real-time PCR detector system (Bio-Rad, Richmond, CA, USA) according to Pérez-Izquierdo *et al.* (2019). Two 2017 root samples from girdled plots were not extracted due to lack of sample material at this stage. Tests with known amounts of plasmid DNA and corresponding M13 primers (Pérez-Izquierdo *et al.*, 2019), using the same PCR conditions, indicated no significant PCR inhibition by the root extracts.

Hyphal production

Ectomycorrhizal (ECM) fungal hyphal production over the growing season (same dates as root bags) was estimated using sand-filled ingrowth bags (Wallander *et al.*, 2013). 5 x 5 cm nylon mesh bags with a 37 µm mesh size were filled with 18 g of sand from Lake Torneträsk (Parker *et al.*, 2015). The sand was sieved to select particle sizes between 0.125 and 1 mm and autoclaved twice, then dried at 100 °C for 72 hours. Bags were designed to be thinner than common practice (only 0.5 cm thick when filled) to limit the distance that mycorrhizal fungi had to grow in order to colonise the sand, and to encourage fungal groups that may not typically grow into sand to colonise (Hagenbo *et al.*, 2018). Bags were inserted into the ground at a 45° angle, directly below the litter layer, 30 cm from each CO_2 efflux collar but on the opposite side to the root ingrowth cores. Prior to insertion, bags were wetted with deionised water on a solid surface in order to ensure uniform sand depth across the bag. Bags remained in the field over the same period as the root bags and blanks were maintained in the laboratory. Sand was extracted from bags four to six hours after recovery from the field, frozen at -80 °C, and freeze-dried for 72 hours in a ModulyoD freeze drier (ThermoFisher Scientific, Waltham, MA, USA). 1.5 g of sand from each bag were sonicated in 25 ml of deionised water for 10 minutes in order to free hyphae from the sand. A 10 ml aliquot of the hyphae-containing solution was transferred to a Falcon tube, to allow further separation of hyphae and sand by sedimentation, then transferred into an open container, dried at 50 °C, weighed and analysed for C content using a Flash Smart™ elemental analyser. This process was repeated for eight blank samples that had not been deployed in the field and the average C content was subtracted from all samples.

Statistical analysis

The effects of girdling, species (willow or birch) and season (early, mid and late) on soil CO_2 efflux, soil moisture and temperature were analysed using linear mixed effects models with the nlme package in R (Pinheiro *et al.*, 2016; R Development Core Team, 2016). In the linear mixed effects model, “plot” was designated as a random variable, to account for the paired design of the experiment, as was “sub-plot”, to take account of repeated measures. Soil CO_2 efflux immediately after girdling treatment in June 2017 was not considered in the analysis, as it was assumed that the treatment had not yet taken effect. All flux data were natural-log transformed in order to conform to the assumptions of the parametric analysis. The effect

of girdling on root and hyphal production, LAI, canopy NDVI and understorey NDVI in birch and willow plots was also analysed using linear mixed effects models after natural-log transformation when appropriate (except NDVI, which required arcsine-square root transformation in order to be appropriate for parametric analysis).

Results

Across all plots, birch had significantly higher soil CO_2 efflux rates than willow plots in 2017 (P = 0.005; Fig. 2) but not in 2018. Girdling significantly reduced soil CO_2 efflux in both 2017 (P < 0.001; Fig. 2) and 2018 (P < 0.001; Fig. 2) in birch and willow plots compared to paired control plots. This reduction in soil CO_2 efflux was large and sustained throughout the peak seasons of 2017 and 2018. The effect of girdling was maintained into late season (September), although not as pronounced then as in mid-season. The girdling treatment did not have a detectably larger effect on soil CO_2 efflux in the birch plots compared to the willow plots in either year, with a statistically non-significant interaction term between species and treatment (P = 0.38 and P = 0.11 in 2017 and 2018, respectively; Fig. 2).

The girdling treatment allowed for the estimation of 'canopy-linked' soil CO_2 efflux (the difference between control and girdled plots) as a proportion of the total soil CO_2 efflux in the control plots (Fig. 2, Fig.S2). The remaining proportion of the total flux constituted respiration of free-living heterotrophs and remaining roots (understorey and canopy species roots that were still alive). Over the 2017 growing season, the average contribution from canopy-linked sources to total soil CO_2 efflux in the birch plots was 33 %, but this increased markedly to 53 % during the peak growing season in early August (Fig. 2, Fig. S2). In 2018 the average canopy-linked contribution to soil CO_2 efflux was again 33 %, with a maximum in early August of 46 %. The canopy-linked contribution to soil CO_2 efflux in willow shrub plots was smaller, but still considerable, with an average of 26 % (in 2017) and 21 % (2018), and maximum contributions of 38 % and 30 %, peaking in early August in each of the respective years.

Girdling significantly reduced total root production compared to control plots in 2017, for birch and willow combined (willow: -30 % change, birch: -75 % change; P = 0.009; Table 2, Fig. 3), with no significant difference between species (P = 0.834). This difference was lost in 2018, with no significant effect of species or girdling treatment on root production. However, girdling caused a highly significant reduction in birch ITS copy numbers in ingrowth bags in 2018 (P = 0.004, Table 2, Fig. 5), with birch root production decreased to almost zero in girdled plots. Girdling also tended to reduce birch copy numbers during the first growing season of the treatment (2017) (P = 0.079, Table 2, Fig. 5).

Although birch control plots tended to have higher hyphal production than girdled plots or willow plots in 2017, there was no overall effect of girdling and only a marginally significant difference between birch and willow plots ($P = 0.059$; Table 2, Fig. 4). By 2018, however, there was a highly significant effect of girdling on hyphal production owing 99 % reduction in girdled birch plots ($P < 0.001$; Table 2, Fig. 4). The lack of difference between girdled and control in 2018 in willow plots was associated with a significant interaction between treatment and species ($P < 0.001$).

Willow plots had significantly lower NDVI than birch canopy, despite no significant difference in LAI (Table 2), likely due to the pubescent leaves of *S. lapponum*, which reduce reflectivity (Street *et al.*, 2007). Girdling significantly reduced canopy NDVI of both species, but more so in the willow plots, resulting in a significant interaction between species and treatment (Table 2). In 2017, despite differences in canopy NDVI, LAI remained unaffected by girdling with no differences between species. In 2018, LAI in girdled birch plots (0.65 m^2 m^{-2}) dropped significantly below control values (0.92 m^2 m^{-2}) due to reduced birch leaf development in girdled plots ($P = 0.024$, Table 2). Understorey NDVI under birch was the same between girdled and control plots in both 2017 and 2018, on average remaining at 0.77. The average LAI of the understorey of 0.88 m^2 m^{-2}, estimated from NDVI according to the relationship between ground vegetation LAI and NDVI at a 3 m scale at a nearby site (LAI = 0.00059 $e^{9.502\ NDVI}$ (Williams *et al.*, 2008)), indicated that birch trees contribute approximately half of the leaf area in this ecosystem. It was not possible to make this calculation in the willow plots due to the resolution of the imagery, making it hard to differentiate willow and understorey from the drone platform.

Soil moisture varied significantly between vegetation types ($P < 0.001$, Fig. S3b). In the growing season of 2017, soil moisture was 1.6 times higher in willow plots than in birch plots and in 2018 it was 1.5 times higher. There was no statistically detectable effect of girdling on soil moisture in either year. Both willow and birch plots were exposed to a flush of water at the time of snow melt in May/June, but soon after soil moisture dropped to distinctly lower levels in birch plots until soil freeze-up in November (Fig. S3b). Soil temperature was not different between species or girdling treatment (Fig. S3a).

Discussion

Mountain birch forests and willow shrub patches are amongst the most productive ecosystems in the Fennoscandian subarctic and are representative of plant communities that are expanding onto tundra as northern latitudes warm (Myers-Smith *et al.*, 2011; Hofgaard *et al.*, 2013). Although expansion of forest and shrub communities is expected to increase gross primary productivity there is little understanding of how vegetation change will influence the C dynamics of the whole system, primarily because the subsequent fate of assimilated C is so poorly quantified and understood (Street *et al.*, 2018). Here, we use a girdling experiment to show that recently fixed C contributes 53 % and 33 % (peak season and full season, respectively) to soil CO_2 efflux in mountain birch communities, and 38 % and 26 % to soil CO_2 efflux in willow communities. The results suggested that much of the C fixed into these relatively productive ecosystems is rapidly returned to the atmosphere, constituting a significant fraction of soil CO_2 efflux.

We found that leaf area of the birch canopy (measured here at 0.5-0.92 $m^2\ m^{-2}$ depending on sampling year) was approximately the same as the leaf area of the understorey (~0.88 $m^2\ m^{-2}$ based on conversion from NDVI). The understorey of subarctic (Kulmala *et al.*, 2019) and boreal (Wardle *et al.*, 2012) forests can contribute 50 % of GPP, and exclusion of all autotrophic C inputs to the soil in a subarctic birch forest (both canopy and understorey) resulted in a ~50 % reduction in soil CO_2 efflux at peak growing season (Hartley *et al.*, 2012). We therefore hypothesised that the contribution from canopy assimilation to autotrophic soil CO_2 fluxes in mountain birch would reflect its contribution to community leaf area, which would equate to an approximate 25 % reduction following girdling given the broadly equal LAI of overstorey and understorey vegetation. Thus, our finding of a 33 % reduction in soil CO_2 efflux during the growing season following cessation of inputs from only the birch canopy disagrees with our hypothesis and suggests that birch makes a larger than expected contribution to soil CO_2 fluxes. In the wider context of autotrophic-heterotrophic soil CO_2 efflux partitioning (broadly 50 % autotrophic (Subke *et al.*, 2006)), the relative contribution of one species which is only one half of the ecosystem leaf area is also remarkable.

The peak season 53 % reduction in soil CO_2 efflux with girdling in early August roughly coincides with peak vegetation productivity (Heliasz *et al.*, 2011). Although

phenology of peak belowground allocation will vary from year to year, we suggest that allocation belowground scales with increasing assimilation aboveground. The scale and seasonality of the canopy-driven soil efflux agrees closely with the results of a previous girdling experiment in a Swedish boreal forest (Högberg *et al.*, 2001). The Högberg *et al.* (2001) study was carried out in Scots pine forest (*Pinus sylvestris*) with a sparse understorey and approximately double the density of trees compared to the present study. Although there are obvious differences between these ecosystems, our data suggest that mountain birch trees play a disproportionate role in controlling below-ground C dynamics in these ecosystems. A girdling treatment in an ericaceous dwarf shrub community (*Calluna vulgaris*) showed no detectable change in soil CO_2 efflux (Kritzler *et al.*, 2016), indicating that roots and associated fungi made a much smaller contribution to total soil respiration than in mountain birch forest. Should trees or shrubs expand onto ericaceous heath, our experiment suggests that the autotrophic component of soil CO_2 efflux would increase disproportionately along with increased GPP.

The reduction in soil efflux of CO_2 in birch plots after girdling coincided with reductions in production of birch roots and mycorrhizal mycelium in birch plots, demonstrating the tight coupling between C assimilation in the canopy, belowground biomass production and return via soil CO_2 efflux. The reduction in birch root and mycelium production was greatest in the second year of the treatment with four of six girdled plots showing zero or near-zero biomass production. This delayed effect suggests that these trees have a degree of resilience to disturbance, potentially in the form of stored non-structural carbohydrates that can supplement rhizosphere demand in the short term (Palacio *et al.*, 2008), also supported by some resprouting of shoots below the girdling line. Nevertheless, it is clear that reduction in C supply from the canopy to the rhizosphere resulted in large reductions in soil respiration in both birch and willow plots.

In 2018, despite the large reduction in birch ITS copy numbers, there was no significant difference in total root production in the girdled and control plots. We were not able specifically to estimate ericaceous biomass production directly (because of our lack of primers targeting the Ericaceae). However, the recovery of overall root productivity, coupled to the major decline in birch ITS copy numbers, strongly suggests that there was an increase in root productivity from the ericaceous understorey plants, most likely as a result

of these plants being released from competition with the birch trees. In open birch forests, it is unlikely that shading by the canopy is limiting ericaceous understorey growth; instead, competition for nutrients may exert a stronger control. In support of this explanation, invertebrate herbivore events are known to exert a strong control on canopy productivity in subarctic birch forests (Bjerke *et al.*, 2014) and also increase soil nitrogen availability (Parker *et al.*, 2017), which, along with frass inputs, is suggested to be driven by reduced uptake by the birch canopy (Parker *et al.*, 2017). Such disturbance events may release the understorey from belowground competition and allow for greater ericaceous shrub productivity, as appears to have occurred in our girdling study. Overall, these findings further demonstrate the disproportionate role that birch trees play in driving C and nutrient cycling within these ecosystems, when compared with their contribution to total LAI.

The substantial canopy-linked soil respiration flux integrates a number of processes that occur subsequent to the allocation of photosynthate to the roots. Firstly, roots and their associated mycorrhizal fungi respire as they grow through the soil (Söderström & Read, 1987; Hagenbo *et al.*, 2019). The second potential source of canopy-linked soil CO_2 efflux is positive priming of soil organic matter: greater microbial decomposition of soil C as a result of autotrophic C delivery (Kuzyakov, 2002). Priming has previously been inferred to reduce soil C storage in mountain birch forests compared to tundra heath, despite high above-ground biomass and productivity (Hartley *et al.*, 2012). Furthermore, ECM fungi have been linked to decomposition in boreal, organic-rich soils through the production of extracellular oxidative enzymes (Lindahl & Tunlid, 2015; Sterkenburg *et al.*, 2018; Zak *et al.*, 2019), especially when mineral nitrogen availability is low (Bödeker *et al.*, 2014). Therefore, priming of organic matter by tree and shrub roots and associated mycorrhizal fungi could contribute a significant fraction of the large canopy-linked soil CO_2 efflux.

It is clear that more mycorrhizal hyphae were produced in the birch plots than in the willow plots and that girdling dramatically reduced this production to almost zero. Respiration by mycorrhizal hyphae can contribute from 14 to 26 % of total soil CO_2 efflux in boreal forest (Hasselquist *et al.*, 2012; Hagenbo *et al.*, 2019) and is likely to contribute a significant fraction of the canopy-linked flux in our mountain birch plots. Furthermore, non-melanised mycorrhizal necromass is known to degrade rapidly (Wilkinson *et al.*, 2011; Drigo *et al.*, 2012; Fernandez *et al.*, 2019) and ECM-dominated soils correlate with high soil

turnover rates and low soil C compared to ericoid mycorrhizal-dominated systems (Clemmensen *et al.*, 2015; Parker *et al.*, 2015). Therefore, we expect that, in areas of the tundra where soils are dominated by ECM symbioses, fungal symbionts play an important role in the rapid return of autotrophic C as soil CO_2 efflux.

The girdling experiment demonstrates a significant contribution of the willow shrub canopy to soil CO_2 efflux. At its peak, canopy-linked soil CO_2 efflux in willow plots reached 38 % of the total flux. Willows belong to a genus of shrubs that are well documented to be expanding in the Arctic, garnering significant interest in their associated ecosystem feedbacks (Myers-Smith *et al.*, 2011, 2019b). Shrubby ecosystems in the tundra have previously been linked to fast turnover of below-ground C (Parker *et al.*, 2015; Sørensen *et al.*, 2018) and leaf litter (Demarco *et al.*, 2014; Parker *et al.*, 2018), but with this experiment we were able to quantify soil CO_2 efflux directly driven by recent canopy C assimilation. We hypothesised that girdling would cause a larger relative reduction in soil CO_2 efflux in birch than in willow plots as a result of higher allocation of C to mycorrhizal networks in the former. Indeed it is clear that more mycelium was produced in birch plots, and we observed a trend towards greater canopy-linked soil CO_2 efflux was greater in the birch plots. However, the fact that this was not statistically significant may be related to the more rapid reduction in LAI within the willow plots.

The limited hyphal colonisation of the in-growth bags in both girdled and control willow plots suggests that willow shrubs do not rely significantly on ECM extramatrical mycelium for nutrient acquisition (although colonisation by smooth, contact type ECM fungi without extensive mycelial proliferation outside of the roots may take place (Agerer, 2001)). As outlined above, this may be due to a) greater soil moisture in the willow plots, and potential for anoxic conditions, having adverse effects on the fungi (Lodge, 1989; Wurzburger *et al.*, 2004; Barnes *et al.*, 2018), or b) drifting snow (Naito & Cairns, 2011) resulting in increased influx of dissolved and particulate compounds and/or increased mobilisation of nitrogen by the winter-active microbial community (Nadelhoffer *et al.*, 1991; Schimel *et al.*, 2004), and thus reducing investment in mycorrhizas by the shrubs. We propose that arctic willows, growing typically in moist topographies, may rely more on roots and direct uptake of nutrients, than on ECM fungi.

We have demonstrated that recent photosynthate regulates soil CO_2 efflux in subarctic forest communities beyond what is expected from the contribution of canopies to community LAI. Trees and shrubs are potential future land cover types on what is presently tundra heath (Pearson *et al.*, 2013) and some of the extra C that will be fixed as a result of increasing photosynthesis in these more productive ecosystems will be rapidly returned to the atmosphere through the rhizosphere. Unexpectedly, we found that birch and willow canopies contributed similarly large proportions to soil CO_2 efflux, but much more canopy-fixed C was allocated to mycorrhizal mycelium by birch. At present, our understanding of rhizosphere processes and subsequent C losses lags behind research on above-ground processes. Evidence from previous research suggests that rhizosphere priming of soil organic matter occurs in subarctic treeline forests and that forest expansion could even lead to a net loss of C from the ecosystem (Hartley *et al.*, 2012). The majority of tundra soils have scarce mineral nutrient availability (Shaver *et al.*, 1992), therefore greater investment below-ground by plants may be required to mobilise nutrients for further growth. If soil CO_2 efflux increases in tundra soils in response to increased plant growth, a critical research priority will be to understand what proportion of the increased efflux is short-term root respiration, and how much is the decomposition of soil organic matter in response to rhizosphere inputs.

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764 Table 1: Average (± 1 standard error) vegetation and soil characteristics in control and
765 girdled plots of birch (n = 6 pairs) and willow (n = 5 pairs). Soil respiration values are for
766 measurement days prior to implementation of the girdling treatment.

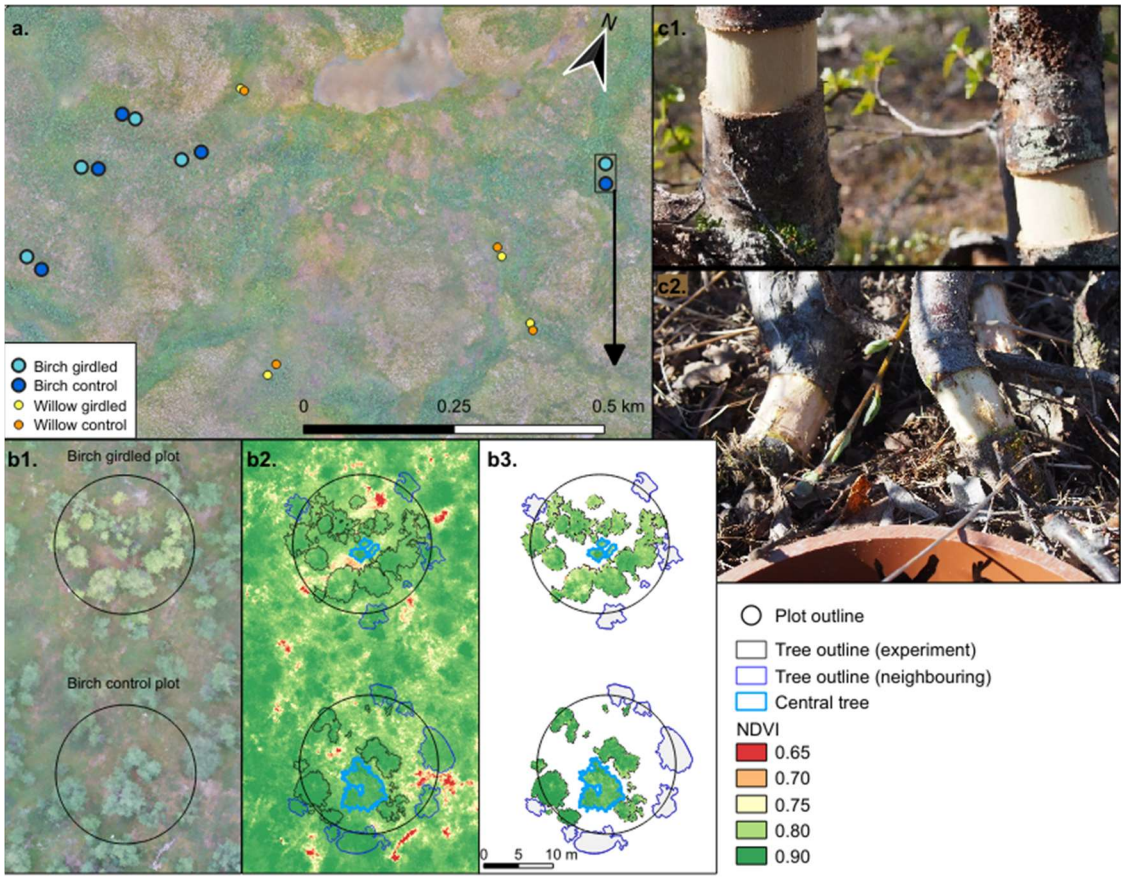
	Birch		Willow	
	Control	Girdled	Control	Girdled
Trees (Trees ha^{-1})	573 ± 72.1	600 ± 83.6		
Stems (Stems m^{-2})	0.27 ± 0.03	0.20 ± 0.01	5.98 ± 0.45	5.52 ± 0.43
Canopy height (cm)			82.7 ± 9.26	76.7 ± 8.57
Organic horizon SOC (kg m^{-2})	2.80 ± 0.17	2.57 ± 0.20	3.07 ± 0.46	2.50 ± 0.35
Soil C:N ratio	29.6 ± 1.02	28.7 ± 1.33	25.0 ± 0.38	25.6 ± 1.09
Soil CO_2 efflux (μmol m^{-2} s^{-1})	2.80 ± 0.15	2.59 ± 0.18	2.48 ± 0.34	2.37 ± 0.10

767

768 Table 2: Mean (± 1 SE) root and hyphae production over full growing seasons, canopy LAI, and understorey Normalised Difference Vegetation
769 Index (NDVI) in late July in birch and willow, girdled and control plots (Birch = 6 paired plots, Willow = 5 paired plots) in 2017 and 2018. Root
770 production values are from the top 6 cm of soil, hyphae production from the top 3.5 cm of soil. Test statistics from linear mixed effects models
771 for fixed effects (species, treatment, and the interaction, if present) for each response variable in each year are provided in line.

	Birch Forest				Willow Shrub											
	Control		Girdled		Control		Girdled		Species			Treatment			Species x Treatment		
2017									d.f.	F	*P*	d.f.	F	*P*	d.f.	F	*P*
Roots (mg C bag^{-1})	11.5 ± 3.79		2.75 ± 0.83		6.30 ± 1.15		4.37 ± 1.27		1,9	0.05	0.829	1,9	11.1	0.009	1,9	2.50	0.148
Birch ITS copies (bag^{-1})	6032 ± 3678		180 ± 116									1,3	6.87	0.079			
Hyphae (mg C bag^{-1})	1.07 ± 0.39		0.19 ± 0.07		0.11 ± 0.03		0.24 ± 0.13		1,9	4.64	0.060	1,9	1.35	0.275	1,9	0.95	0.356
Canopy LAI (m^2 m^{-2})	0.50 ± 0.21		0.58 ± 0.23		1.51 ± 0.50		0.56 ± 0.15		1,9	0.41	0.537	1,9	3.87	0.081	1,9	3.93	0.079
Canopy NDVI	0.87 ± 0.01		0.85 ± 0.01		0.83 ± 0.01		0.77 ± 0.01		1,9	47.41	< 0.001	1,9	122	< 0.001	1,9	32.94	< 0.001
Understorey NDVI	0.81 ± 0.02		0.81 ± 0.01									1,5	0.14	0.724			
2018																	
Roots (mg C bag^{-1})	8.84 ± 2.19		7.15 ± 1.55		17.41 ± 5.79		7.68 ± 2.48		1,9	1.48	0.255	1,9	2.90	0.123	1,9	0.84	0.383
Birch ITS copies (bag^{-1})	30000 ± 25317		18 ± 17									1,5	24.72	0.004			
Hyphae (mg C bag^{-1})	2.43 ± 0.87		0.02 ± 0.01		0.32 ± 0.18		0.18 ± 0.06		1,9	1.30	0.284	1,9	28.86	< 0.001	1,9	21.58	0.001
LAI (m^2 m^{-2})	0.92 ± 0.10		0.65 ± 0.07									1,5	10.33	0.024			
Canopy NDVI	0.81 ± 0.01		0.74 ± 0.01		0.72 ± 0.01		0.65 ± 0.02		1,9	39.92	< 0.001	1,9	68.33	< 0.001	1,9	0.29	0.606
Understorey NDVI	0.74 ± 0.02		0.74 ± 0.02									1,5	0.00	0.964			

772



773

774 Figure 1: (a) Location of paired girdled and control plots of birch (blue circles) and willow
775 (orange circles) at field sites south of Abisko (note that 1 birch pair and 1 willow pair are 200
776 m south, out-with the image). (b) Birch pair 6 with plot perimeters superimposed (b1), false
777 colour imagery of NDVI values and trees within the experiment marked (b2) and
778 experimental and central study tree only marked (b3). (c) Examples of stem girdling in birch
779 plots (c1) and willow plots (c2).

780

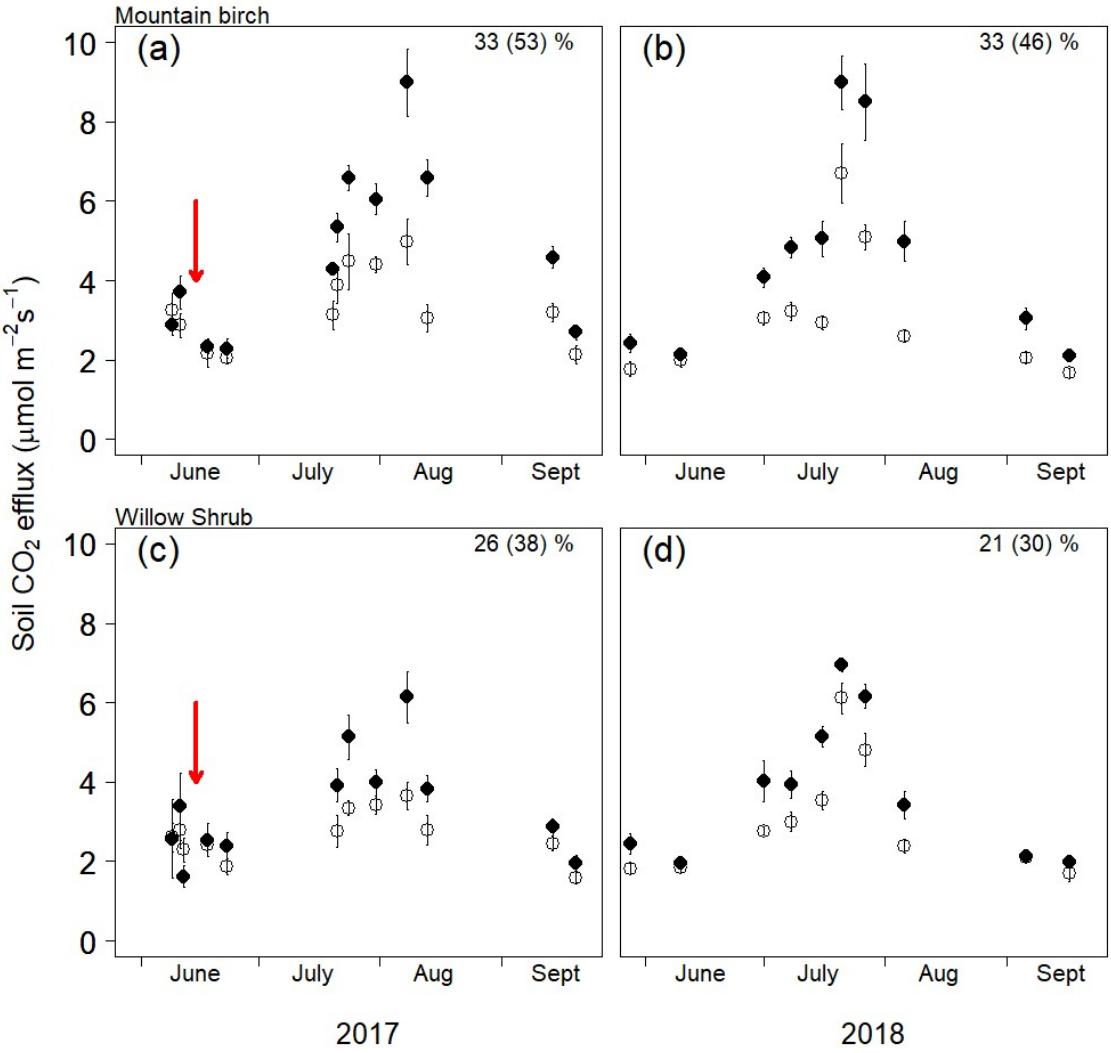


Figure 2: Soil CO_2 efflux from Mountain birch in 2017 (a) and 2018 (b), and Willow shrub in 2017 (c) and 2018 (d) in control (filled circles) and girdled plots (open circles). Points represent mean values at each sampling date (± 1 standard error). Arrows (red) indicate the date of girdling in the respective communities. In 2017 there were significant effects of species ($F_{(1,9)} = 14.0$, $P = 0.005$), girdling treatment ($F_{(1,10)} = 24.3$, $P < 0.001$) and season ($F_{(1,186)} = 130$, $P < 0.001$) and no interactions between species and treatment ($F_{(1,9)} = 0.59$, $P = 0.46$). In 2018 there was no significant effect of species ($F_{(1,9)} = 3.24$, $P = 0.11$) but effects of girdling treatment ($F_{(1,10)} = 36.7$, $P < 0.001$) and season ($F_{(2,196)} = 168$, $P < 0.001$); there was no interaction between species and treatment ($F_{(1,9)} = 2.00$, $P = 0.19$). The percentage contributions of the canopy to soil CO_2 efflux over the over the whole growing season and at its seasonal maximum (in brackets) are reported in the top right of each panel.

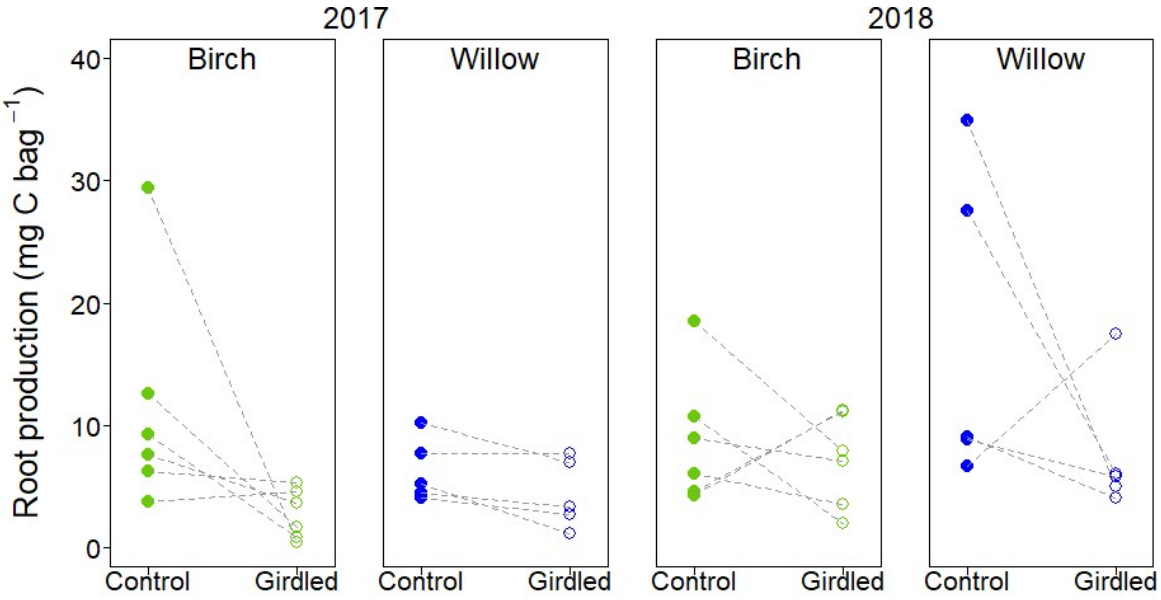


Figure 3: Root production (mass of C into ingrowth bag) in paired (denoted by dashed connecting lines) control and girdled plots in 2017 and 2018 in birch (green) and willow (blue) plots. The results of statistical analyses are shown in Table 2.

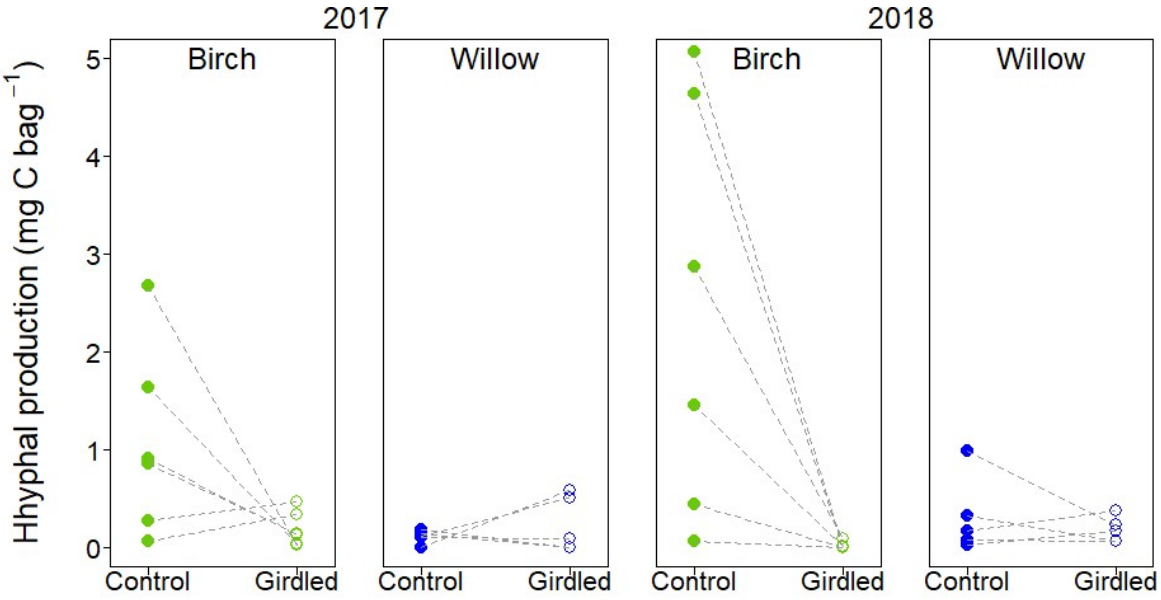
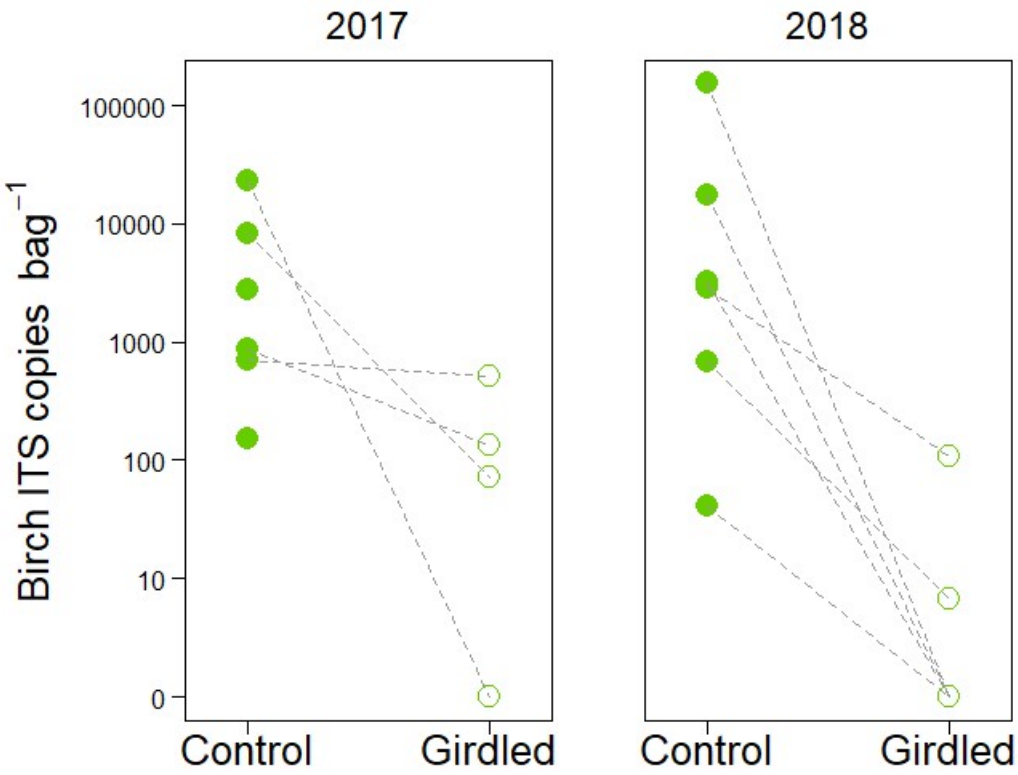


Figure 4: Hyphal production (mass of C into ingrowth bag) in paired (denoted by dashed connecting lines) control and girdled plots in 2017 and 2018 in birch (green) and willow (blue) plots. The results of statistical analyses are shown in Table 2.



803

804 Figure 5: Birch ITS copy numbers in ingrowth bags in paired (denoted by dashed connecting
805 lines) birch girdled and control plots in 2017 and 2018. The results of statistical analyses are
806 shown in Table 2.

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808