# *Effects of soil warming and nitrogen foliar applications on bud burst of black spruce*

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## *Abstract*

The observation of phenological events can be used as biological indicator of environmental changes, especially from the perspective of climate change. In boreal forests, the onset of the bud burst is a key factor in the length of the growing season. With current climate change, the major factors limiting the growth of boreal trees (i.e., temperature and nitrogen availability) are changing and studies on mature trees are limited. The aim of this study was to investigate the effects of soil warming and increased nitrogen (N) deposition on bud burst of mature black spruce (*Picea mariana* (Mill.) BSP). From 2008 onwards, an experimental manipulation of these environmental growth conditions was conducted in two stands (BER and SIM) at different altitudes in the boreal forest of Quebec, Canada. An increase in soil temperature (H-treatment) and a canopy application of artificial rain enriched with nitrogen (N-treatment) were performed. Observations of bud phenology were made during May-July 2012 and 2013. In BER, H-treatment caused an anticipation (estimated as 1-3 days) while N-treatment a delay (estimated as 1-2 days but only in 2012) in bud burst. No treatments effect was significant in SIM. It has been demonstrated that soil temperature and N availability can play an important role in affecting bud burst in black spruce but the effects of these environmental factors on growth are closely linked with site conditions.



***Keywords:*** Boreal forest, bud burst, climate change, nitrogen, *Picea mariana*, soil warming


***Key message:*** **in mature black spruce, bud burst process is anticipated by soil warming while delayed by foliar applications of nitrogen, however the effects depend on growth conditions at the site.**

## *Introduction*

The observation of phenological events can be used as biological indicator of environmental changes (Parmesan, 2007; Richardson et al., 2013), as also revealed by the lengthening of the growing season of plants (Boulouf Lugo et al., 2012; Linderholm, 2006). During the final two decades of last century, the growing season of vegetation lengthened by 6.7 days/decade in North America, primarily because of an early spring resumption of about 4.4 days/decade (Zhou et al., 2001). Compared to long-term observations, experiments of climate manipulation showed discrepancies in the timings of phenological events, probably because of their inability to reproduce the complex plant-environment relations (Wolkovich et al., 2012). Nevertheless, they are a useful tool for understanding the response of ecosystems to climate change (Kreyling and Beier, 2013) and for estimating the ability of forests to produce renewable products and services (Maynard et al., 2014). In particular, long-term and multi-factor experiments seem able to better represent the changed environment by giving plants time to manifest adaptive responses (Rustad, 2008).

In areas with seasonality, the transition from rest to active growth is marked by a well-known event, called bud burst, which, especially at high latitudes, mainly determines the length of the vegetative season. Generally three major factors drive the onset of bud burst: photoperiod, winter and spring temperatures (Laube et al., 2014). The effect of photoperiod is well documented: increasing the daily period of illumination, bud burst is anticipated (Basler and Körner, 2012; Partanen et al., 1998). The accumulation of cold temperature during winter (called chilling) allows the breaking of bud dormancy while the accumulation of hot temperature (forcing) during spring allows the initiation of bud development. The inverse interaction between these two accumulations plays a key role, namely forcing temperature sum decreases with increasing duration of chilling (Fu et al., 2013). With the expected global warming, although the cold temperatures of winter in the boreal regions seem to be sufficient to achieve the chilling requirement (Linkosalo et al., 2008), milder winters could change precipitation patterns with a consequent modification of the thickness of snow on the ground. In particular, because of global warming, an increase of precipitation, particularly in liquid rather than solid form (snow or ice), is expected (Price et al., 2013). In addition, higher spring temperatures could, on the one hand, influence snowmelt and the relative soil thaw, and on the other, force bud burst (Richardson et al., 2013). The observed anticipation of the time of

bud burst could increase the risks from late frosts (Hanninen, 2006) and have consequences with the proliferation of various insects (Zhang et al., 2014).

The coordination between the above- and belowground systems is a prerequisite for tree survival, in particular under a changing climate: leaves guarantee photosynthesis and reproductive functions, while roots provide water and nutrients. Modifications of the conditions in one of these two systems might lead to physiological responses in the other through the long-distance communication by hormonal signals and sugar remobilization (Puig et al., 2012). Although localized heating experiments revealed a local modification in stem and root growth (Begum et al., 2012; Lupi et al., 2012b), the effect on other parts of the plant, including the timings of the bud burst process, appears limited, at least in the short term (Bronson et al., 2009). For technical reasons, the materials used to analyze the responses of tree species to warming experiments are generally juvenile trees, as saplings or seedlings (Rossi and Bousquet, 2014). However, recent studies (Richardson and O'Keefe, 2009; Vitasse and Basler, 2014) revealed a significant discrepancy of the phenological phases between young and adult trees, mainly due to the different ontogenetic stage (Vitasse and Basler, 2013). In addition, the phenological response of a single species to the current global warming is also influenced by the spatial location as site conditions have altered the intra-population genetic variability (Primack et al., 2009).

In an environment where the amount of N available for plants is severely limited by climate (Reich et al., 2006), knowledge of the effects of this element on growth is of crucial importance. Previous soil warming experiments revealed an increase in the rate of nitrogen mineralization (Butler et al., 2012; Campbell et al., 2009; Melillo et al., 2011) and, especially with the current climate change, an increase of this nutrient may affect tree growth. In addition, to overcome the limited availability of nitrogen in the soil, trees have recourse to the absorption of this element directly at the canopy level. With respect to fertilization to the soil, N foliar applications are readily absorbed with retention values higher than 70% (Gaige et al., 2007) and have a more rapid influence on plant physiology (Sparks, 2009).

Changes in N availability may influence the physiology of plants and other related organisms, especially in the long term (D'Orangeville et al., 2013; Houle and Moore, 2008; Rossi et al., 2012). Several studies have analyzed the effect of N on bud burst (Fløistad and Kohmann, 2004; Kula et al., 2012; Lumme and Smolander, 1996; Murray et al., 1994; Sigurdsson, 2001; Thitithanakul et al., 2012), sometimes producing

contrasting results probably related to the different treatment types and doses, or the age of the plants. The use of seedlings is generally preferred because the change in the level of fertilization appears to be less costly and easier than in mature trees. For example, Bigras et al. (1996) analyzed the effect of nitrogen on black spruce seedlings and found no differences in the onset of bud burst between treatments.

Since 2008, an experiment of canopy N-enriched combined with a soil warming treatment applied during the growing season is conducted to evaluate the growth reaction of mature black spruce stand [*Picea mariana* (Mill.) BSP] in terms of tree phenology (Lupi et al., 2010, 2012a; Lupi et al., 2012b). The aim of this study was to investigate the effects of soil warming and increased N deposition on bud burst of mature black spruce. We tested the hypothesis that both soil warming and N deposition can modify bud phenology. The experimental design was replicated in two sites, differing in altitude and in climatic and edaphic conditions, corresponding to a 2 K difference in mean annual air temperature.

## *Materials and methods*

*Study sites*

The study was conducted in two mature, even-aged and monospecific black spruce [*Picea mariana* (Mill.) BSP] stands at different altitudes in the boreal forest of Quebec, Canada (Table 1). The first site (abbreviated as BER) is located near Lake Bernatchez, in the Monts-Valin (48°51' N, 70°20' W, 611 m a.s.l.); the second site (SIM) is at a lower altitude, in the Laurentides Wildlife Reserve, within the Simoncouche research station (48°13' N, 71°15' W, 350 m a.s.l.). As a consequence of forest fires in 1922 in SIM and estimated between 1865 and 1870 in BER, black spruce became the dominant species. The climate is continental, with long cold winters and short warm summers. On average, May-September mean temperature is 11.4 and 13.3 °C, and total rainfall is 453.5 and 376.3 mm, in BER and SIM respectively (Lupi et al., 2010; Rossi et al., 2011). Snow cover lasts from November to May, reaching a depth of up to 130 cm in BER and 110 in SIM. Complete snowmelt occurs 20 days earlier in SIM [day of the year (DOY) 120] than in BER (DOY 140). Due to the cold environment and the consequent limited period without snow, BER has a growing season about 15 days shorter than at SIM (Boulouf Lugo et al., 2012).

*Experimental design*

The experimental design at the sites was constituted of a 60x60 m square plot, divided in twenty-five 12x12 m squares, of which only 12 acting as experimental units and the others being buffer zones. One tree was chosen in each experimental unit from among those with upright stem and healthy overall appearance. From 2008, two treatments were performed: an increase in soil temperature (H-treatment) and a canopy application of artificial rain enriched with nitrogen (N-treatment). The combination of treatments produced four experimental groups: heated (H), N-enriched (N), heated and N-enriched (HN) and control (C) trees. Each treatment was applied to three trees: in total, 12 trees were studied per site (Lupi et al., 2010).

For the H-treatment, heating cables were installed during autumn 2007 at about 20-30 cm depth, where the root system of black spruce is mainly localized (Ruess et al., 2003). The cables followed a spiral pattern at a distance of 90–200 cm from the stem collar, leaving 30 cm between coils of the cables. Heating increased the soil temperature by 4 °C during the growing season (April-July), the period in which most cambial

activity takes place (Thibeault-Martel et al., 2008), in accordance with the estimates for 2050 of the FORESTEM climatic model (Houle et al., 2012). The energy required for heating was supplied by a diesel generator placed near the sites. The difference in temperature was maintained with measurements of soil temperature at 1–2 m from the stem collected between the coils of the cables in three heated and three non-heated points per site. The generator automatically warmed the soil when the differences between treated and control trees were lower than 4 K. In order to counterbalance any root and soil disturbance during installation, non-heating cables were installed around the C and N trees, using the same procedure,.

For N-treatment, sprinklers were installed above the canopy of each tree and, once a week during the frost-safe period (June-September), the equivalent of 2 mm of rainfall was applied over a circular area of 3 m radius centered on the stem. C and H trees were irrigated using a water solution with chemical composition similar to local natural rainfall (Duchesne and Houle, 2008), while HN and N trees were irrigated with a threefold increase in ammonium nitrate ($NH_4NO_3$) concentration. The N increase is about 50% more than the current natural level (0.7-1 kg $ha^{-1}$ $year^{-1}$).

*Air and soil temperature*

A standard weather station was installed in a forest gap close to the experimental plot of each site to measure air temperature (°C). Measurements were taken every 15 min and data were stored as hourly averages in CR10X dataloggers (Campbell Scientific Corporation, Edmonton, Canada). Soil temperature was measured at about 1–2 m from the stem between the coils of the cables in three heated and three non-heated points per site. Measurements were taken at the same time interval as those of air temperature and stored in CR1000 dataloggers.

*Bud burst*

Observations of bud phenology were made during May-July 2012 and 2013, corresponding to the $5^{th}$ and $6^{th}$ year after the beginning of the experiment. Each experimental tree was visited weekly and several photos (35×, 54× and 140× zoom) of the crown were taken at two different expositions (North and South) with a compact digital camera (Canon PowerShot SX40 HS, Canon Inc., Tokyo). The photos were analyzed in the lab for determination of the bud phenology phases according to the modified procedure by

Numainville and Desponts (2004) (Table 2 and Figure 1). The modification consisted of dividing the shoot elongation phase into three additional phases (phases 6-8 in Table 2) to better discriminate the bud burst process. Phase 1 was considered as the beginning of bud burst. Observations stopped when all individuals reached phase 8. Since bud burst on a tree takes place with different timings, the percentage of canopy reaching a certain phenological phase was also estimated through visual interpretation of the photos.

*Statistics*

The succession of the bud burst phases showed a non-linear trend, so different methods of linearization of the data were tested (Sit and Poulin-Costello, 1994). The one producing the best results was transformation of the type $x' = \frac{1}{x}$, and $y' = y^2$, where *x* and *y* represent DOY and the phenological phases, respectively. For each year and study site, the treatments effect on bud burst was tested using a covariance analysis (ANCOVA) applied using JMP 9.0 (SAS Institute, Cary, NC). Goodness-of-fit of each regression involved the proportion of variation accounted for ($R^2$) and the distribution of residuals. A preliminary analysis showed no statistical difference between the two exposures, so the data collected in the North- and South-exposed part of the canopy were considered as repetitions within the same individual. The predictions by the ANCOVA models were graphically represented by applying the inverse transformations on the results, which gave a clearer representation of bud burst.

## *Results*

*Air and soil temperature*

Daily air and soil temperature showed different absolute values but similar trends between the two sites (Figure 2). In 2012, air temperature rose abruptly until 21-23 °C in mid-May (DOY 145-147), and subsequently decreased at the end of May and during the first days of June (DOY 148-162). After this period, new warming was observed with temperatures fluctuating between 10 and 25 °C for the rest June and July (DOY 163-200). In 2013, air temperature increased gradually during the monitored period, although variations of 10 K within 2-4 days were still observed. Daily averages of air temperature never dropped below freezing point during the studied period (Figure 2). In general, air temperature was 2-3 K warmer in 2012 than 2013, except during July (DOY 185-200), which was hotter in 2013.

Soil temperature gradually increased in both years with some daily fluctuations, but the variations were lower than those observed for air temperature. In 2012, measured temperatures were higher, especially during May (DOY 145-150). With the exception of the first 5 days (2013) in the control trees in BER, soil temperature was always above 5 °C. Overall, the soil in BER was 3-5 K colder than in SIM. The warming system was able to maintain the difference in soil temperature between H and C trees within a narrow range of values, between 3.5 and 5.2 K, more frequently between 4.0 and 4.5 K. Overall, the differences in soil temperature between H and C trees showed higher fluctuations in BER than in SIM.

*Bud burst process*

The dynamics of the process of bud burst was clearly non-linear (Figure 3): the first phases [from 0 (bud completely closed) to 4 (leaves are visible)] took place in a short time while the latter phases [from 5 (internal scales broken) to 8 (elongated shoot with almost or fully open needles)] required more time for completion. In this study, the last phases required a longer time since they consider the macroscopic emergence of needles from the bud scales (i.e. elongation of the leaves) whereas the early phases relate to the physical and morphological changes of the bud. For these reasons, different methods of linearization were tested and the one that more suitably represented the data was chosen. This statistical procedure allowed trends among the various applied treatments to be adequately compared and analyzed.

The dynamics of change of the phenological phases were correctly detected by the ANCOVA models, as revealed by the significant effect of DOY ($p<0.001$, Table 3). The general trend, which is represented in Figure 3 by applying the inverse transformations on the predictions of the model, showed a rapid initial growth in the first five phases (approximately the first fortnight), followed by a slowdown (approximately the last 15 days), producing an exponential pattern.

The use of linear transformation has allowed us to obtain good values of fitting ($0.82 \leq R^2 \leq 0.91$) and a small difference between the observed and estimated values (Figure 4), especially in the case of those with high frequencies.

*Treatments effect*

Bud burst occurred an estimated 1-3 days earlier in H-trees, but the effect was significant only in BER with $p<0.01$ and $p<0.001$ in 2012 and 2013, respectively (Table 3). The interaction H×DOY was also significant in 2012 in BER. N-treatment exhibited a significant effect in 2012 in BER, with N and HN trees showing delayed bud burst compared to the trees receiving not-enriched rain (C and H trees). The delay produced by N-treatment was estimated as 1-2 days. No treatments effect was significant in SIM.

On average, bud burst began and finished earlier in SIM than in BER. In BER, the onset and ending of bud burst occurred, on average, earlier in 2012 (DOY 155 and 187) than in 2013 (DOY 159 and 195) (Table 4). Furthermore, the time spent completing the process of bud burst was shorter in 2012 (32 days) than in 2013 (35 days). In SIM, the onset of bud burst didn't change much between years, a mean date of DOY 150 being estimated with variations limited to one day. In addition, the ending of bud burst occurred earlier in 2012 (DOY 183) than in 2013 (DOY 189) and, consequently, the duration of bud burst was approximately 6 days longer in 2013 than in 2012.

## *Discussion*

In the present study, we tested whether an increase in soil temperature and in N deposition can influence the process of bud burst in mature individuals of black spruce of two boreal stands. The hypothesis that both soil warming and N deposition can modify bud burst phenology was only partially accepted. Changes in bud burst were observed only in the colder site (BER) in response to the considered environmental parameters: soil warming anticipated bud phenology while N application delayed the bud burst process. The treatments were not significant in the warmer site (SIM).

*Soil temperature and bud burst*

Soil warming significantly modifies the timings of bud burst but only in the colder site with a deeper layer of mosses. In BER, the onset of bud burst in H-trees occurred 1-3 days before the control, revealing a possible interaction between roots and shoot in the early spring. We propose that these results could be explained by the effect of temperature on water absorption and through water and solute (including mineral nutrients, sugars and hormones) transport from roots to shoots.

First, low temperatures prevent the absorption of water by the roots (Alvarez-Uria and Korner, 2007; Bergh and Linder, 1999), inhibiting or delaying the physiological processes of plants. Thus, at high latitudes the time of soil thaw, and consequently the availability of water in liquid form for roots, plays a key role in the resumption of vegetative growth (de Fay et al., 2000; Rossi et al., 2011). The increase in soil temperature in spring, which has been observed to cause earlier root activity (Lupi et al., 2012b), also anticipates water uptake and the onset of trunk sap flow (Repo et al., 2008). According to Greer et al. (2006), the advancement of bud phenology may be explained by an early mobilization of water and mineral nutrients. Furthermore, xylem sap flow carries cytokinins (CKs), a fundamental root-to-shoot link signal (phytohormone) mainly produced in root tips that stimulates the breaking of bud dormancy (Domagalska and Leyser, 2011; Sachs and Thimann, 1967). In addition, CKs supplied by the roots are limited at suboptimal temperatures, while warming may increase their production or concentration in sap, promoting the onset of bud burst (Lyr, 1996) with the anticipation of water sap flow.

Although in the short-term a previous study revealed that a 5 °C increase in the soil temperature does not significantly affect the onset of bud burst and the shoot growth of 12 years old black spruce trees, from the

third year of treatment the final shoot length showed an increase in the order of 14% (Bronson et al., 2009). Similarly, Lupi et al. (2012b) found that xylogenesis is influenced by soil warming during the third year of treatment, anticipating its onset by 6 days. Since the advancement of the bud burst depends on the level of increase in soil temperature (Lopushinsky and Max, 1990), it seems that the effect requires a period of adaptation for trees. However, the effect of the treatments on bud burst was not considered in the years prior to this study, so it cannot be excluded that some changes had taken place previously.

Significant effects of soil temperature on bud burst were found in BER, the site at higher altitude with shorter snow-free periods and growing seasons (Rossi et al., 2011). In this site, temperature is a more limiting factor and so the plants may be more sensitive to small increases. As confirmation of this, the different seasonal pattern of temperature in the two years influenced the onset of bud burst, which was earlier in the year when spring temperatures (of both soil and air) were higher. *Vice versa*, SIM is a warmer site at the lower edge of the closed black spruce forest distribution, where snowmelt usually occurs in early May (DOY 120) and the growing season is consequently longer (Rossi et al., 2011). Independently of year, bud burst started on average on DOY 150 and soil warming didn't affect the onset. Considering the similar photoperiod between the two sites, air temperature probably plays a predominant role in determining the initiation of bud development in SIM, confirming the finding of Bronson et al. (2009). Overall, independently of study site, the bud burst process lasted longer in 2013 than 2012, 3 and 6 days more on average at BER and SIM, respectively. It is likely that the low temperatures recorded in 2013 extended the development time.

The genetic differentiation among populations is the result of local adaptation of the tree species to different environmental factors (usually climate, edaphic characteristics and parasites), as a consequence of the differential selection pressures (Savolainen et al., 2007). In addition, a single genotype can create different phenotypes (phenotypic plasticity), which are the result of the ontogenetic changes following the integration of the environmental information (Sultan, 2000). Since the magnitude of the variation of the considered environmental factors is the same between the two populations, it is reasonable to assume that this phenotypic plasticity has influenced the response of the studied plants. As evidence of this, a recent study realized in these two sites (Rossi, 2014) shows how environmental factors, including in particular

temperature, play a fundamental role in determining the sensitivity of the bud burst process in different provenances of black spruce.

*Nitrogen and bud burst*

N-treatment significantly affected the timings of bud phenology, but only in BER in 2012, which showed a negative effect with a delay in bud burst (1-2 days). Studying the effects of stress caused by fertilization on Silver beach, Kula et al. (2012) found that bud burst was delayed in plants submitted to increasing N-inputs. According to Omarov et al. (1999), this delayed bud burst is due to the fact that the surplus of ammonium nitrogen limits the production of CKs and, simultaneously, increases that of abscisic acid. However, that experiment differed from ours by the high amount of N applied and type of application, with the N solution watered directly onto the soil. Despite the limited concentrations of N applied to the canopy, changes have been observed at root level (Lupi et al., 2012b): in the third year of treatments, the general trend of the onset of growth revealed a delay in trees treated with nitrogen (HN and N trees). Nitrogen applications directly to the canopy possibly altered nitrogen content or produced chemical modifications in buds that delayed the start of their development. In particular, three hormones (auxin, strigolactones and CKs) seem to be key factors in bud development, the first two with inhibitory functions (Puig et al., 2012). Although there is a strong interdependence between nutrients and phytohormones, their ability to influence each other is still not completely clear (Kiba et al., 2011).

As for the warming, site conditions may have amplified the treatment effect: in SIM, N content is higher in soil and needles (Lupi et al., 2012b), so it may be deduced that N is more available than in BER. In addition, the amount of N needed for plant growth is closely linked to the dry weight production (Krouk et al., 2011). Because of less favorable growth conditions, trees show lower growth in the colder site (BER) and it is possible that an addition of N has a greater effect.

## *Conclusion*

Our innovative experimental design demonstrated that soil temperature and N availability can play an important role in affecting bud burst in black spruce. It is well known that soil temperature is influenced by the air: although soil warms and cools more slowly, the trend is comparable with air temperature, revealing

a strong interdependence. In this context snow characteristics, given its importance in the boreal forest during the resumption of the growing season, perform an important role as an interface between these two environments, favoring or limiting the exchange of heat. In North America, global warming resulting in decreased snow permanence on the ground and earlier snowmelt is strongly related to local year-to-year temperature trend and snow depth (Peng et al., 2013; Rossi et al., 2011). Although soil temperature requires several years to manifest an effect, it remains a crucial factor to consider when evaluating the effect of climate change on tree growth. The delaying effect of N fertilization on bud burst, unlike temperature, has not revealed a stable response but seems to be most influencing in the long term. The attempt to find a physiological explanation to the results of this study is suitable as a basis for future research, which will allow to verify and better understand the interaction between environmental signals and phenology, and consequently productivity, of trees. These knowledge's are still scarce especially at the level of mature stage. Finally, the effects of these environmental factors on growth are closely linked with the site conditions, revealing a greater effect where they represent a major limiting factor.

## *Author contribution*

HM coordinated the project. HM, AD and SR conceived the idea and the experimental design. SR and DDB developed data analysis. DDB wrote the manuscript with the help of the other co-authors.

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## *Conflict of interest*

The authors declare that they have no conflict of interest.

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## *Caption list*

**Table 1:** Stands characteristics and chemistry of the organic soil layer of the studied plots.

**Table 2:** Phases of bud burst according to Numainville and Desponts (2004) modified.

**Table 3:** F-statistics of the ANCOVA models comparing bud burst between treatments (H = heated treatment, N = N-enrichment treatment) and DOY.

**Table 4:** predicted dates (DOY) of bud burst onset (phase 1) and ending (phase 8) of control (C), heated (H), heated and N-enriched (HN), N-enriched (N) trees in 2012 and 2013 for the two sites. The duration is reported in parentheses.

**Figure 1:** Examples of the eight phenological phases of bud burst according to the description presented in table 1. As the photos of the developing buds were taken from the ground (between 20-25 m from the growing bud) the resolution of the pictures could be suboptimal because digital zoom was used (140× digital zoom) was used when the optical zoom of the camera was exceed.

**Figure 2:** daily air and soil temperature and difference in soil temperature between control and heated treatment during 2012 and 2013.

**Figure 3:** dates of bud burst predicted by ANCOVA models for the different treatments [control (C), heated (H), heated and N-enriched (HN), N-enriched (N) trees]. The models were performed on linearized distributions but are represented by applying the inverse transformations.

**Figure 4:** residuals of the ANOVA models performed on dates of bud burst detected in trees subjected to different treatments [control (C), heated (H), heated and N-enriched (HN), N-enriched (N) trees]. The size of dots is related to the percentage value estimated for each phenological phase in the different sampling days, namely, the larger the point, the greater the number of buds in the given phase-day (between 0 and 100%).

**Table 1**

| | BER | SIM |
|---|---|---|
| Tree age[1] | 133.40 ± 2.72 | 80.60 ± 1.52 |
| Height (m) [1] | 16.83 ± 0.82 | 17.22 ± 0.75 |
| DBH (cm) [1] | 20.38 ± 1.82 | 20.92 ± 1.42 |
| Stand basal area ($m^2$ $ha^{-1}$) [1] | 32.5 | 32.5 |
| C:N [2] | 50.2±8.7 | 39.1±9.6 |
| N organic soil ($g \cdot kg^{-1}$) [2] | 8.5±0.1 | 9.7±1.1 |

[1] Stand data from Rossi et al. (2014).
[2] Organic soil layer from Lupi et al. (2012b).

## Table 2

| Phase | Characteristics |
|---|---|
| 0 | Bud completely closed. |
| 1 | Visible opening of bud. The bud scales begin to separate. No bud lengthening visible. |
| 2 | Apparent elongation of the internal bud scales. The diameter of the bud is less than that of the crown of outer scales. |
| 3 | Swollen bud, whose diameter exceeds that of the crown of outer scales. The inner scales are still opaque, no leaves visible. |
| 4 | Leaves are visible through the translucent inner scales. |
| 5 | Internal scales broken. Leaves still closed on the axis of the shoot, which is still stocky and rounded. |
| 6 | Limited shoot elongation with little or non-visible leaves. |
| 7 | Shoot elongation with visible and opening needles. |
| 8 | Elongated shoot with almost or fully open needles. |

**Table 3**

| | BER | | SIM | |
|---|---|---|---|---|
| Source of variation | 2012 | 2013 | 2012 | 2013 |
| DOY | 1847.93*** | 2158.03*** | 983.40*** | 2129.44*** |
| H | 8.50** | 11.50*** | 1.60 | 2.42 |
| N | 4.43* | 0.12 | 0.16 | 1.31 |
| H×N | 0.20 | 0.17 | 0.06 | 0.84 |
| H×DOY | 4.31* | 1.5 | 0.02 | 0.01 |
| N×DOY | 0.59 | 0.01 | 0.71 | 0.31 |
| H×N×DOY | 0.47 | 0.01 | 0.02 | 0.05 |

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

**Table 4**

| | BER | | SIM | |
|---|---|---|---|---|
| | 2012 | 2013 | 2012 | 2013 |
| C | 155-186 (31) | 160-196 (37) | 150-182 (32) | 151-190 (39) |
| H | 152-187 (35) | 159-193 (34) | 150-183 (33) | 151-189 (38) |
| HN | 154-187 (32) | 159-193 (34) | 150-184 (34) | 149-188 (39) |
| N | 156-188 (31) | 160-196 (37) | 149-183 (34) | 151-190 (39) |

**Figure 1**

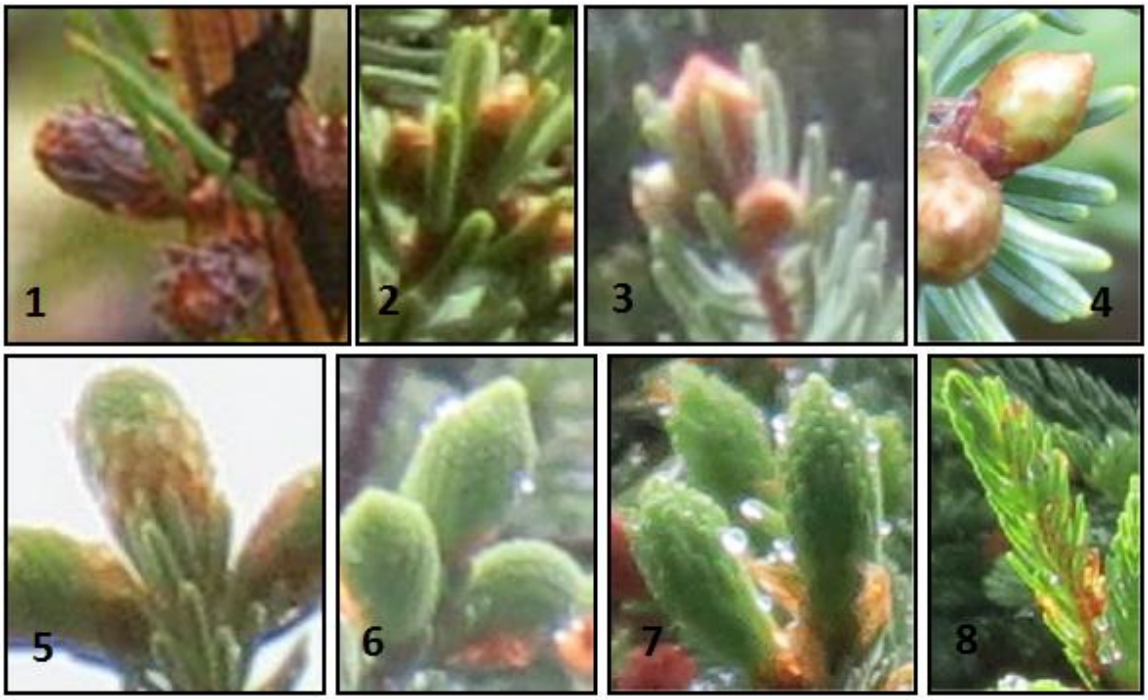


**Figure 2**

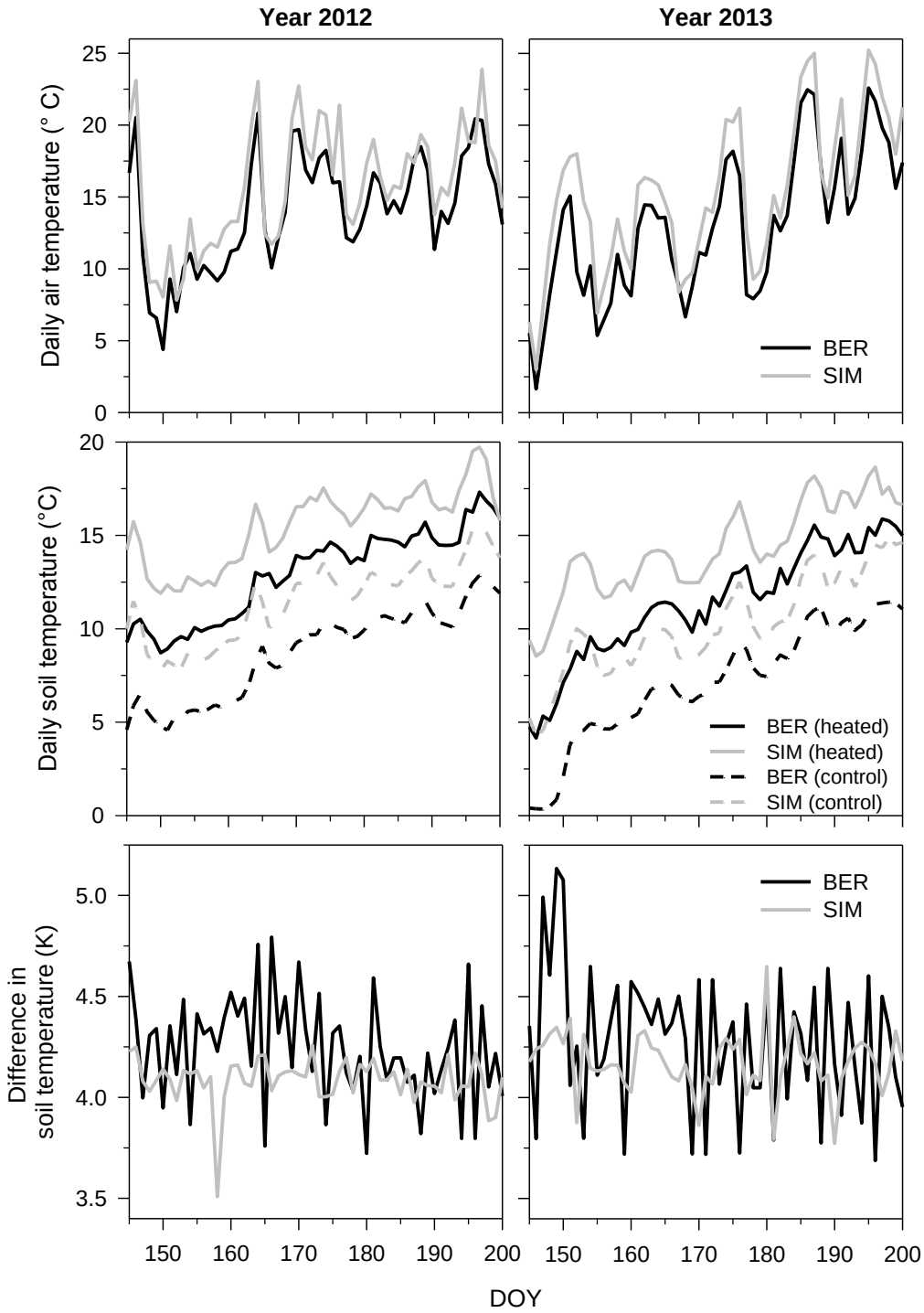

Year 2012
Year 2013
Daily air temperature (° C)
Daily soil temperature (°C)
Difference in soil temperature (K)
BER
SIM
BER (heated)
SIM (heated)
BER (control)
SIM (control)
DOY

**Figure 3**

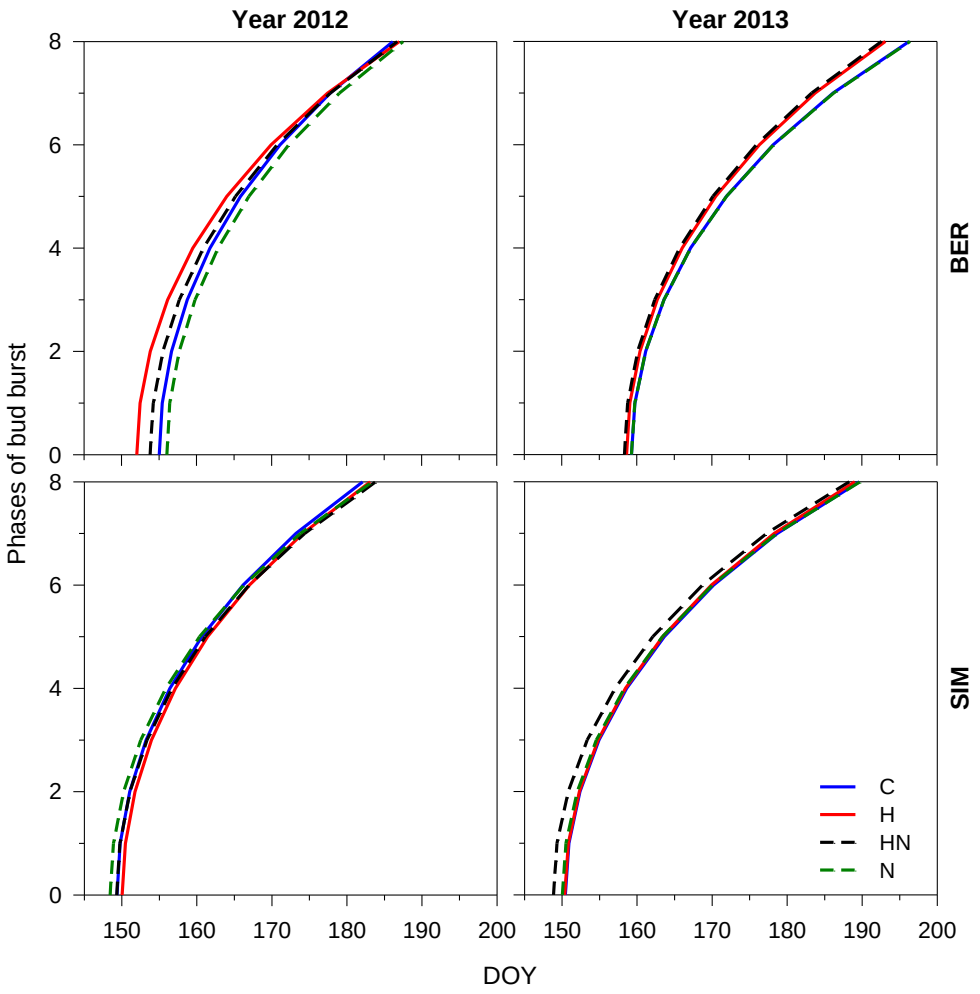
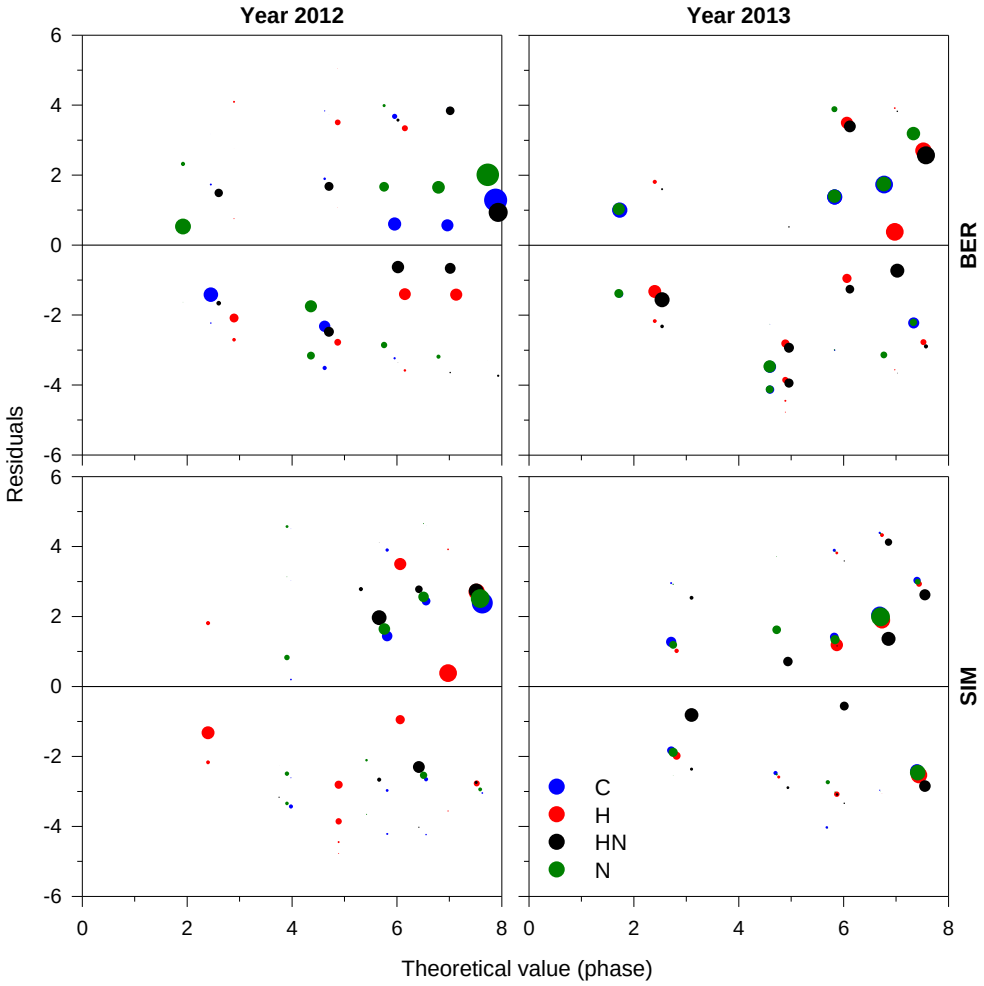

Year 2012
Year 2013
BER
SIM
Phases of bud burst
DOY
C
H
HN
N
0
2
4
6
8
150
160
170
180
190
200

**Figure 4**


Year 2012
Year 2013
BER
SIM
Residuals
Theoretical value (phase)
C
H
HN
N