Permafrost-driven differences in habitat quality determine plant response to gall-inducing mite herbivory

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Summary

1. Canada's northern boreal forests are undergoing rapid change associated with warming trends, with permafrost thaw leading to increased forest stress and compositional changes in plant communities. Within this context, the influence of commonly occurring, resident natural enemies on plant species remains poorly understood. One of the dominant deciduous shrub genera, *Betula*, is abundant in northern forest–wetland landscapes, and is highly susceptible to leaf galling by mites, but the impacts of these specialist arthropod herbivores on plant-level physiology remain unknown.

2. We examined the impacts of mite galling on a suite of ecophysiological traits in *Betula glandulosa*, a shrubby species commonly found on permafrost plateaus, bogs and fens in a boreal wetland–forest mosaic, and on two congeners, *B. occidentalis* and *B. neoalaskana,* found only on plateaus.

3. *B. glandulosa* leaves on plateaus showed marked declines in photosynthetic capacity [A_{max}], stomatal conductance [g_s] and transpiration [E] in response to galling. However, *B. glandulosa* leaves in bogs and fens did not respond similarly, implying strong habitat-related differences in response to galling herbivory. The impacts on plateaus were reinforced by similar responses of *B. occidentalis* and *B. neoalaskana* leaves to galling, and confirmed that on plateaus, galling appears to have significant negative effects on photosynthetic uptake. Moreover, carbon uptake in infested leaves showed photosynthetic declines even at low galling intensity. Interestingly, neighbouring nongalled leaves adjacent to galled ones showed similar declines in gas-exchange rates in all the three species found on plateaus. However, reduced stomatal conductance and transpiration in galled and neighbour leaves did not affect whole plant water status, as we found no differences in either midday or diurnal stem water potentials between galled and gall-free stems in any of the species.

4. Synthesis: Based on these findings, we suggest that the impacts of mite galling are variable, and are likely to be most pronounced in habitats and on individuals that are already exposed to abiotic stresses such as the cooler, but more variable soil temperatures experienced by individuals on permafrost plateaus. Furthermore, galling can potentially contribute to localized thaw processes in these environments via impacts on plant functioning.

Key-words: *Betula*, bog, boreal, eriophyid, fen, gas-exchange, photosynthesis, plant–herbivore interactions, Taiga, water potential

Introduction

Northern boreal forests are changing rapidly in response to climate warming and some of the most rapid warming is occurring in North-western North America (Johannessen *et al.* 2004). The most dramatic manifestation of this warming is the accelerated rate of thaw of permafrost (ground perennially at or below 0 °C) (Camill 2005), which has lead to marked decreases in the spatial cover of permafrost in the discontinuous zone where permafrost occurs as patches below landscape (Hinzman *et al.* 2005; Quinton, Hayashi & Chasmer 2011). Such changes have substantial ecological implications because permafrost plateaus form the physical foundation for most forest cover in this region and as such climate warming and permafrost thaw lead to reduced habitat availability and increased abiotic stress for many forest species. Much of the boreal forest biome is underlain by discontinuous permafrost, thus understanding the ecological implications of permafrost thaw is critical.

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Climate warming is expected to increase biotic stress in high latitude ecosystems by increasing the winter survival of arthropods (Bale *et al.* 2002). Prior work has focussed mainly on sporadic outbreaks of 'pest' species that are expected to increase in frequency with global warming in boreal forests at lower latitudes (Volney & Fleming 2000; Kurz *et al.* 2008; McKenzie, Peterson & Littell 2008). Much less attention has been given to the influence of prevalent resident non-outbreak arthropods (especially at high latitudes) that are likely to gain importance as forests become increasingly climate stressed. Given the rapid change occurring at the southern margin of the discontinuous permafrost, understanding the influence of resident natural enemies with respect to forest composition, productivity or health and how this may change with further warming and permafrost thaw is important for understanding the trajectory of these systems.

One such group of specialist arthropods is the gall-inducing guild of insects and mites found on vegetation canopies worldwide. Gall inducers disrupt host plant function in a number of ways, including modifications to plant-level architecture (Larson & Whitham 1997), damage to vegetative and reproductive buds (Dorchin, Cramer & Hoffmann 2006; Raman 2007), reductions in plant growth (Fay, Hartnett & Knapp 1996), down-regulation and reallocation of plant defence compounds (Nyman & Julkunen-Tiitto 2000) and modifications to leaf physiological function. An important and relatively well-studied aspect of gall inducer–plant interactions is their influence on photosynthesis, which can range from compensatory increases (e.g. Dorchin, Cramer & Hoffmann 2006) to marked decreases (e.g. Larson 1998). However, a recent and growing body of literature seems to suggest that galling for the most part causes significant declines in photosynthesis (e.g. Zvereva, Lanta & Kozlov 2010; Patankar, Thomas & Smith 2011; Nabity *et al.* 2012). There is increasing evidence that gall formation causes disruption beyond simple stomatal down-regulation, and in fact has significant negative impacts on photosystem II quantum efficiency (Aldea *et al.* 2006; Huang *et al.* 2011), along with modifications to photosynthetic pigments (Yang *et al.* 2003; Gailite, Andersone & Ievinsh 2005; Samsone, Andersone & Ievinsh 2012) and carbon metabolites (Chakrabarti, Chakrabarti & Chakrabarti 2011; Patankar *et al.* 2013). Such physiological impacts have been linked with reduced radial growth rates in trees, indicating reduced productivity at the whole plant level in response to gall-mite herbivory (Patankar, Thomas & Smith 2011).

Host response to galling, however, is not uniform and can differ significantly depending on several factors including habitat quality (Goncalves-Alvim, Collevatti & Fernandes 2004), differential plant species responses (Aldea *et al.* 2006; Samsone, Andersone & Ievinsh 2012), host plant ontogeny (Patankar, Thomas & Smith 2011), individual plant quality (Egan & Ott 2007) and growing conditions (i.e. low resource stress; Fay, Hartnett & Knapp 1993, 1996), and thus generalizations based on point samples from either a single species or habitat type may not reflect variations associated with host response to galling attack. For example, host plant quality (Egan & Ott 2007) and resource availability (Fay, Hartnett & Knapp 1996) can play a large role in determining levels of gall infestation and host plant physiological response to galling respectively. Indeed, host plants subjected to increased environmental stress tend to fare poorly in response to galling attack (e.g. Fay, Hartnett & Knapp 1996). Thus, there is a need to look at galling impacts across several related species and across distinct habitats in heterogeneous landscapes to derive meaningful inferences on the functional importance of galling on host plant health, productivity and long-term survival. Furthermore, in ecosystems experiencing rapid change in structure and community composition due to climate stress, natural enemies may benefit from changes and contribute to positive feedback mechanisms of stress, leading to 'stress complexes' often associated with outbreak species (see McKenzie, Peterson & Littell 2008).

Here we examine the response of leaf-level photosynthetic processes to mite galling in three birch (*Betula*) species, and across three characteristic habitats in a nutrient-poor boreal forest–wetland complex in North-western Canada where recent climate warming–related land cover changes have occurred rapidly in recent decades (Quinton, Hayashi & Chasmer 2011). In this system, *Betula occidentalis* Hook and *B. neoalaskana* (Sarg.) occur exclusively on elevated plateaus underlain by permafrost whereas *B. glandulosa* (Michx.) occupies all habitat characteristics of the landscape mosaic at the study site (see site description in Methods). Leaves of all three species are infested with galls (Fig. 1) formed by a single species of eriophyoid mite (*Vasates olfieldi* Amrine & Stasny; Acari: Eriophyidae; F. Beaulieu, personal communication) thus providing an ideal experimental system to examine species- and habitat-related responses to mite-gall herbivory. Our research objectives were fourfold: (i) Determine whether there are systematic differences among habitats (bogs, fens and plateaus) in host plant gas-exchange response to leaf galling, (ii) Examine whether congeneric species co-occurring on a single habitat, show comparable physiological responses to mite galling, (iii) Determine if galling has similar impacts on adjacent neighbouring leaves, or whether they instead exhibit compensatory increases in carbon uptake as seen in some other plant species, and (iv) Examine the relationship between galling intensity (proportion of leaf area galled) and photosynthesis across multiple congeneric host plant species.

Materials and methods

STUDY SITE

The study was conducted in the lower Liard River valley at Scotty Creek (61°18′ N, 121°, 18′ W), Northwest Territories, Canada. Scotty Creek falls occupies discontinuous permafrost, supports peatland complexes of permafrost plateaus and ombrotrophic flat bogs and channel fens (Quinton, Hayashi & Pietroniro 2003) with contrasting edaphic, vegetative features (summarized in Table 1). The ice-rich permafrost underlying the plateaus displaces the plateau ground surface ~1–2 m above the surrounding wetlands (Robinson & Moore 2000); as such the plateaus are relatively well drained (Quinton, Hayashi & Pietroniro 2003; Table 1). Permafrost plateaus support *Picea mariana*-dominated forests, a shrub layer consisting of *Betula*
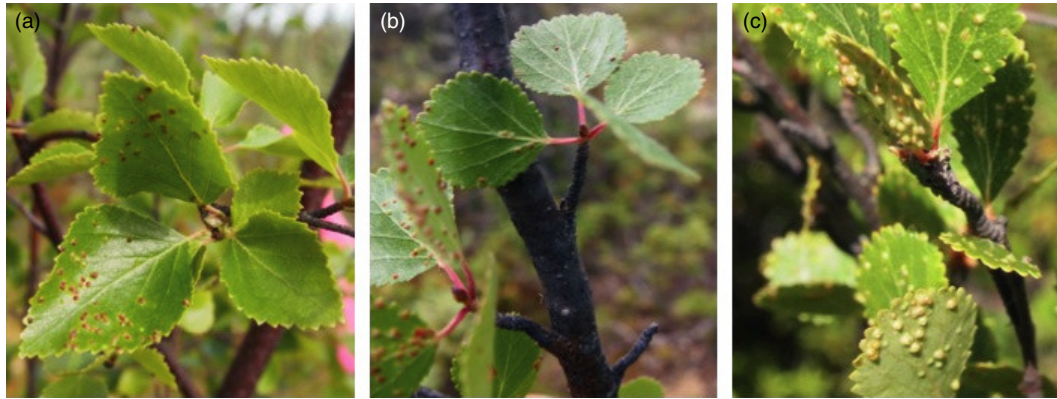
Fig. 1. Leaves galled by eriophyid mites on (a) *Betula neoalaskana*, (b) *B. occidentalis* and (c) *B. glandulosa* during the 2012 summer growing season from permafrost peat plateau sites at Scotty Creek, Northwest Territories. Ungalled neighbouring leaves from the same stalk can be seen adjacent to gall-infected *B. neoalaskana* and *B. occidentalis* leaves.

Table 1. Mean (±SE) characteristics of the three major habitat types included in the study. Plateaus are the only habitat of the three to be underlain by permafrost

Habitat Variable	Plateau	Flat Bog	Channel Fen
%C*	44.68 ± 0.57^b	47.36 ± 0.45^a	48.72 ± 0.51^a
%N*	0.80 ± 0.15^{ns}	0.91 ± 0.07^{ns}	1.20 ± 0.20^{ns}
C:N*	69.63 ± 15.48^{ns}	52.42 ± 3.39^{ns}	43.35 ± 7.89^{ns}
LOI*	96.53 ± 1.80^{ns}	97.19 ± 0.27^{ns}	94.12 ± 1.02^{ns}
Bulk density*	0.04 ± 0.01^{ns}	0.05 ± 0.01^{ns}	0.05 ± 0.02^{ns}
Porosity*	80.65 ± 2.94^{ns}	84.59 ± 10.66^{ns}	72.16 ± 5.86^{ns}
Vol. SWC (10 cm)	0.50 ± 0.06	0.70 ± 0.16	nd
Vol. SWC (20 cm)	0.70 ± 0.05	0.79 ± 0.01	nd
Vol. SWC (30 cm)	0.70 ± 0.01	$nd^‡$	nd
Vol. SWC (40 cm)	0.69 ± 0.01	nd	nd
ALT	42.2 ± 2.2^b	$+200^{†a}$	$+200^{†a}$

Variables are as follows:%C and%N, total carbon and nitrogen as a per cent of mass; C : N, carbon to nitrogen ratio; LOI, per cent loss on ignition; Bulk Density (g cm^{-3}) and Porosity (%) for the upper 20 cm of soil; volumetric soil moisture (Vol. SWC) measured at four depths continuously from June – September 2007; ALT, active layer thickness (maximum seasonal thaw depth, cm). Univariate analysis of variance was used to test for differences in measured site variables. LOI, bulk density, porosity and ALT failed to meet ANOVA assumptions and transformations failed, thus we used Kruskal–Wallis tests to assess habitat-related differences. Lower case superscripts indicated the results of Tukey's *post hoc* analyses.
*From Kenward (2010).
†All measurements of late-July active layer thickness exceeded the 200 cm steel probe used.
‡No data.

and *Rhododendron* species and a ground cover layer of many lichen and bryophyte species. Flat bogs have a roughly 3 m deep organic layer and woody vegetation here can range from none to low shrubs, particularly *Betula*, *Kalmia*, *Chamaedaphne* and *Salix*. Channel fens form the basin drainage network and receive water from adjacent bogs and plateaus. Floating vegetation mats in fens support a range of grasses, sedges and shrubs, including *Betula* species. Within the context of this forest–wetland complex, we define 'soil' as simply the organic layer that plants root in, whether floating on water or elevated on permafrost plateaus. The climate at Scotty Creek is characterized by short dry summers and low annual precipitation, with most precipitation coming in the form of snow during the long winters.

STUDY SPECIES

At Scotty Creek, three species of *Betula* are prevalent: *B. neoalaskana*, *B. glandulosa* and *B. occidentalis*. The three species coexist on permafrost plateaus whereas *B. glandulosa* is also found in bogs and fens. *Betula glandulosa* is the most abundant of the three species, often forming mono-dominant canopies in large patches across permafrost plateaus. All three species are targeted by what has been identified morphologically as a single species of gall-inducing mite (*Vasates olfieldi*) resulting in white to red bulbous protuberances on the adaxial surface of leaves (Fig 1). Among individuals, there is substantial variation in the extent of galling ranging from gall free to heavy galling on much of the plant. Because of the passive dispersal of the mites (Michalska *et al.* 2010), similar patterns of variable galling can be found among branches within a single canopy and between neighbouring leaves.

STUDY DESIGN AND MEASUREMENTS

The study had two components, both of which rely on the natural variability among and within individuals in galling intensity. All measurements were made during 20 July–4 August 2012 at the peak of the growing season.

Component 1: Habitat differences in galling impact on B. glandulosa

Within each habitat (plateau, bog and fen) we selected five galled and five gall-free plants (fen *n* = 2 plants/treatment). We measured physiological traits in (i) gall-free leaves on gall-free plants (hereafter control), (ii) galled leaves on galled plants (hereafter galled), and (iii) gall-free leaves on galled plants that were directly adjacent to galled leaves (hereafter neighbour). Leaves from gall-free plants acted as controls whereas neighbouring leaves on galled plants were included

to test whether galling resulted in compensatory increases in physiological activity in ungalled neighbouring leaves.

Component 2: Within-habitat congeneric comparison of galling impacts

To understand how consistent physiological response was to galling among species, we used the two additional birch species described above, *B. neoalaskana* and *B. occidentalis*. For this part of the study, only *B. glandulosa* plants from plateaus were examined to avoid potential confounding effects due to variation in habitats. The sampling design for this experiment was similar to the *B. glandulosa* habitat comparison study except that we were only able to sample two plants/treatment for *B. neoalaskana*. All leaf measurements were made on fully expanded, sun-exposed leaves from the outer 'canopy' (free from other forms of herbivory or damage), thereby minimizing physiological differences due to shading or timing of bud break. The same leaf condition treatments were measured in the two additional *Betula* species. Within a species, comparably sized individuals were selected to avoid potential confounding effects of ontogeny.

LEAF-LEVEL PHYSIOLOGICAL MEASUREMENTS

Four leaves from each of the five plants per 'treatment' (two plants per treatment in the case of fen *B. glandulosa* and plateau *B. neoalaskana*) were selected for measurements to account for any within-plant variation. For each leaf, maximum light-saturated photosynthetic capacity (A_{max}; μmol m^{-2} s^{-1}), stomatal conductance (g_s; mmol m^{-2} s^{-1}) and transpiration (E; mol m^{-2} s^{-1}) were measured using an infrared gas analyser (LI-6400 XTR portable photosynthesis system; LI-COR, Lincoln NE, USA) at a saturating light intensity of 1000 μmol m^{-2} s^{-1} and at a constant reference CO_2 concentration of 400 μmol m^{-2} s^{-1} (at 50–70% relative humidity and between 20 and 25 °C). Instantaneous water use efficiency (*WUE*) was calculated as A_{max}/E (μmol C/mol H_2O), the ratio of net carbon gain per unit water loss. F_V/F_M (representing potential dark-adapted quantum efficiency of photosystem II [PSII]) was measured on two leaves/plant per treatment (dark adapted for a minimum of 10 min) using an OS1p-modulated fluorometer (Opti-Sciences, Tyngsboro MA, USA). Leaves were collected and transported to a field laboratory where leaf areas, number of galls and total galled area were measured.

STEM WATER POTENTIALS

Galled and gall-free *B. glandulosa* stems (four per plant; bog and plateau: $n = 5$ plants/treatment; fen: $n = 2$ plants/treatment, 10 stems per plant) were collected around noon and midday stem water potentials were measured in the field laboratory using a pressure chamber (Model 1000, PMS Instrument Co., Albany OR, USA) to examine changes in whole plant water status as a consequence of galling. To examine potential diurnal differences in stem water potential, stem psychrometers (PSY1 Stem Psychrometer, ICT International, Armidale, NSW, Australia) were installed on the main stem of one galled and ungalled *B. occidentalis* and *B. neoalaska* ($n = 2$ per species) and water potential measurements were recorded at 30-min intervals for a period of 2–3 days from 1st to 3rd August 2012 providing a continual record of stem water potential. Briefly, a suitable position was located on the main stems of individual plants and the bark tissue and cambium layers were carefully removed using a blade to expose the xylem tissue layer. The stem psychrometer was then

attached to the exposed conducting xylem, and silicon grease was used to seal around the psychrometer and cover any exposed xylem tissue. The psychrometer and adjacent stem were then covered in bubble wrap and an outer layer of aluminium foil to insulate against ambient thermal fluctuations and to reflect direct radiation respectively. The psychrometer was attached to a standalone data logger (ICT International, Armidale, NSW, Australia). The stems of *B. glandulosa* were too small to accommodate the sensors, thus only pressure chamber measurements are available for this species.

PHOTOSYNTHESIS–GALLING INTENSITY RELATIONSHIP

Galling intensity, defined as per cent leaf area occupied by galls, was calculated using image-editing software (Photoshop CS 5, Version 15.0.0, Adobe Systems Inc., San Jose, USA). A Pearson correlation was then applied between leaf-level galling intensity and net photosynthesis, to examine whether or not photosynthetic uptake changed with increasing galling intensity as a function of species or habitat type.

STATISTICAL ANALYSES

For the habitat component, a complete randomized block design was used to examine differences in gas-exchange parameters with leaf condition (galled, neighbour and control) and habitat (bog, plateau and fen) as predictors using individual plants as the unit of replication. Gas-exchange values measured on multiple leaves on a plant were averaged to obtain a mean plant-level value. Within each habitat, the two treatments (galled and gall-free neighbour leaves) from a galled plant and control leaf from a nearby gall-free plant were used as blocks to control for sources of variation between galled plants and their paired controls. Furthermore, the plant number was included as a random factor to account for the fact that two leaves in each block came from the same plant. Where significant, Tukey's HSD multiple comparison tests were then used to determine differences among groups. We used separate blocked one-way ANOVAS to examine differences in gas-exchange parameters among leaf conditions separately for the three *Betula* species (similar to the habitat comparison described above), and applied *post hoc* Tukey's HSD multiple comparisons to test for differences. Similarly, a one-way ANOVA was used to determine stem water potential in *B. glandulosa* galled vs. ungalled stems from the three habitats (JMP 9.0, SAS Institute, Carey, NC, USA).

Results

HABITAT ASSOCIATIONS OF BETULA GLANDULOSA

Leaves showed significant differences in gas-exchange traits in response to galling (Table 2); however, these patterns differed as a function of habitat type (Fig 2). Furthermore, there were significant interactions between habitat type (bog, fen and plateau) and leaf condition (galled, ungalled neighbour and control leaf) for A_{max} ($F_{2,27} = 3.552$, $P = 0.027$; Table 2). On plateaus, galled and neighbouring ungalled leaves showed significant declines in A_{max} (Fig 2a), g_s (Fig 2b) and E (Fig 2c) when compared with control leaves, but this did not translate into differences in instantaneous *WUE* because of similar reductions in both A_{max} and E (Fig 2). No significant

Table 2. Results (df, *F* and *P*) of the randomized block design model testing the main effects of habitat type (plateau, bog and fen), leaf condition (galled, neighbour and ungalled) and their interaction on maximum photosynthetic capacity (A_{max}), stomatal water conductance (G_S), transpiration (E), instantaneous water use efficiency (WUE) and dark-adapted quantum efficiency (F_V/F_M) in leaves of *B. glandulosa* plants in Scotty Creek, Northwest Territories. Significant effects (at $P < 0.05$ level of significance) are highlighted in bold

	df	*F*	*P*
A_{max}			
Habitat	2	6.96	**0.015**
Leaf condition	2	1.71	0.209
Habitat × Leaf condition	4	3.55	**0.027**
g_S			
Habitat	2	6.34	**0.019**
Leaf condition	2	5.34	**0.015**
Habitat × Leaf condition	4	0.45	0.768
E			
Habitat	2	8.59	**0.008**
Leaf condition	2	5.08	**0.018**
Habitat × Leaf condition	4	1.51	0.241
WUE			
Habitat	2	0.28	0.762
Leaf condition	2	2.37	0.122
Habitat × Leaf condition	4	2.22	0.107
F_V/F_M			
Habitat	2	7.56	**0.003**
Leaf condition	1	23.69	**<0.001**
Habitat × Leaf condition	2	4.24	**0.026**

differences were detected in gas-exchange traits among treatments in the bog or fen environments (Fig).

Dark-adapted quantum efficiency of PSII (F_V/F_M) was significantly different between a) leaf conditions ($F_{2,24} = 23.691$, $P < 0.001$) and b) between habitats ($F_{2,24} = 7.56$, $P = 0.003$). Furthermore, there was a significant interaction term ($F_{2,24} = 4.24$, $P = 0.026$). Overall, gall-free leaves from all three habitats showed lower F_V/F_M compared with galled leaves, but this was significant only in fen gall-free leaves (Fig 2e).

No significant differences were detected in midday stem water potential between galled and ungalled *B. glandulosa* stems irrespective of habitat ($F_{1,20} = 1.687$, $P = 0.1974$; Fig 3a), suggesting that galling effects do not extend to whole plant water relations. Stems from bogs had the lowest water potentials, whereas those from fens had the highest ($F_{2,20} = 5.825$, $P = 0.0042$), but this difference was due to the relative difference in water potentials between galled stems from the fen and gall-free stems from bogs (Fig 3a). Regardless of habitat, there was no evidence of midday water stress in either treatment.

PHYSIOLOGICAL RESPONSES TO GALLING IN THREE BIRCH SPECIES

Galled and neighbouring ungalled leaves in all three species of *Betula* showed declines in A_{max} compared with gall-free leaves from control plants, these declines being significant in
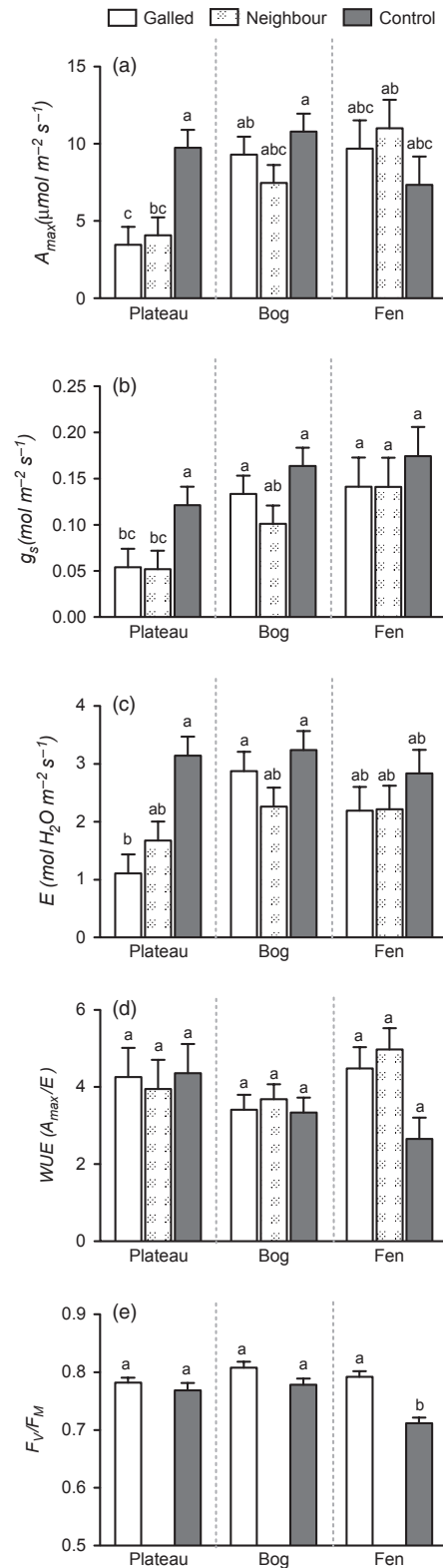

Fig. 2. Net photosynthetic capacity *Amax* (a), stomatal conductance *gs* (b), evapotranspiration *E* (c), instantaneous water use efficiency *WUE* (d) and dark-adapted PS II quantum efficiency (e) measured in galled, neighbouring ungalled and 'control' leaves in three habitats (plateaus, bogs and fens) from Scotty Creek, Northwest Territories. Different letters indicate a significant difference between groups at $P < 0.05$ (complete randomized block design followed by Tukey's multiple comparison tests). Bars are mean + 1 SEM.
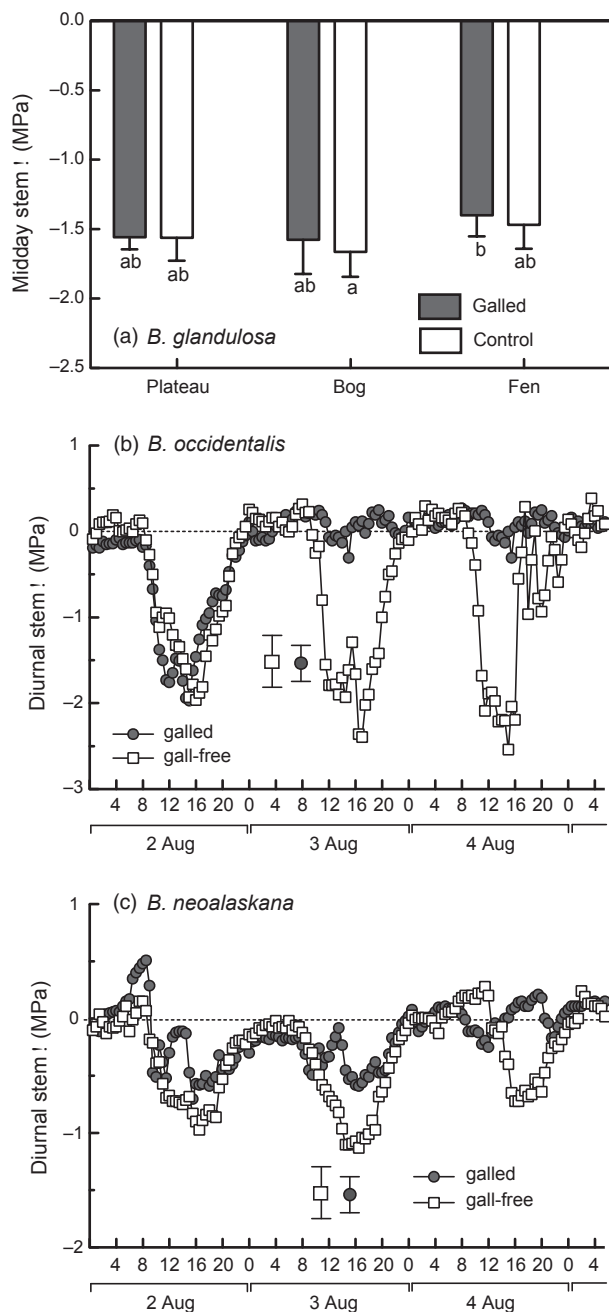
Fig. 3. Midday water potentials (mean + 1 SEM) in galled (n = 20) and ungalled (n = 20) *B. glandulosa* stems on plateaus, bogs and fens (a), diurnal stem water potentials in galled and ungalled *B. occidentalis* (b) and *B. neoalaskana* (c) plants from Scotty Creek, Northwest Territories. For *B. glandulosa*, different letters indicate a significant difference between groups at $P < 0.05$ (ANOVA followed by Tukey's multiple comparison tests). Diurnal measurements were made over a 3-day period 2–4 August 2012. In panels b and c, filled circle = galled and clear square = gall-free midday values (mean ± 1 SEM) from plateau *B. glandulosa* stems. Note that in panel b, symbols for midday values (mean ± 1 SEM) have been shifted slightly to avoid overlap with the diurnal values.

two of them (*B. glandulosa*: $F_{2,12}$ = 7.78, P = 0.013; *B. occidentalis*: $F_{2,9}$ = 5.83, P = 0.039; Fig 4a). Stomatal conductance was highest in gall-free and lowest in neighbouring leaves, this difference being significant in *B. glandulosa*

($F_{2,12}$ = 14.22, $P < 0.001$; Fig 4b). Transpiration was higher in gall-free leaves of *B. glandulosa* and *B. neoalaskana*, with a significant difference found in the former species (*B. glandulosa*: $F_{2,12}$ = 24.11, $P < 0.001$; Fig 4c). There were no detectable differences in *WUE* in any of the species (Fig 4d). F_V/F_M did not differ significantly between galled and gall-free leaves ($F_{1,18}$ = 0.53, P = 0.47) or among the three species of *Betula* ($F_{2,18}$ = 0.51, P = 0.60; Fig 4e). Diurnal patterns of water potential were similar in galled and gall-free stems in both *B. occidentalis* (Fig 3b) and *B. neoalaskana* (Fig 3c) confirming the results from the midday water potential measurements for *B. glandulosa* across habitats. Furthermore, these diurnal measurements show no indication of water stress at any point in the day and both galled and gall-free plants were able to return to a fully hydrated state each evening (Fig. 3b,c).

PHOTOSYNTHESIS-GALLING INTENSITY RELATIONSHIP

A_{max} did not change as a function of galling intensity in any of the study species (*B. glandulosa*: Pearson r = 0.504, P = 0.496; *B. neoalaskana*: Pearson r = −0.380, P = 0.752; *B. occidentalis*: Pearson r = −0.779, P = 0.221; Fig 5).

Discussion

HABITAT-DRIVEN DIFFERENCES IN GALLING RESPONSE IN BETULA GLANDULOSA

Mite galling had significant impacts on gas-exchange rates on permafrost plateaus, resulting in marked declines in maximum photosynthetic capacity (A_{max}; 51.4%), stomatal conductance (g_s; 44.5%) and transpiration (E; 58%) in galled leaves in comparison with leaves from gall-free plants. However, in contrast to the above findings on permafrost plateaus, galled leaves in both bogs and fens showed similar gas-exchange rates as control leaves indicating clear habitat-related differences in response to galling herbivory. To our knowledge, there has been no prior investigation of habitat-related physiological responses to leaf galling herbivory. The only other studies examining habitat-related plant responses have focussed on the impacts of a stem gall inducer on leaf physiological responses (Fay, Hartnett & Knapp 1993, 1996). The response (or lack thereof) of bog and fen leaves to galling, in particular, represents a finding that is novel to what has been previously documented regarding generalized plant responses to galling herbivory. Given this, important questions arise: what are the likely causal mechanisms or processes that underlie habitat-related differences in herbivory response? Specifically, what is it about permafrost plateau environments that make *B. glandulosa* individuals occurring there more responsive to mite-galling herbivory? Differences in surrounding environmental conditions (including abiotic resources and biotic interactions) could provide clues on differences in functional response across habitats. Plant water status (drought-stressed versus well-hydrated tissues), for example, has been known to influence host plant response to galling (Fay,
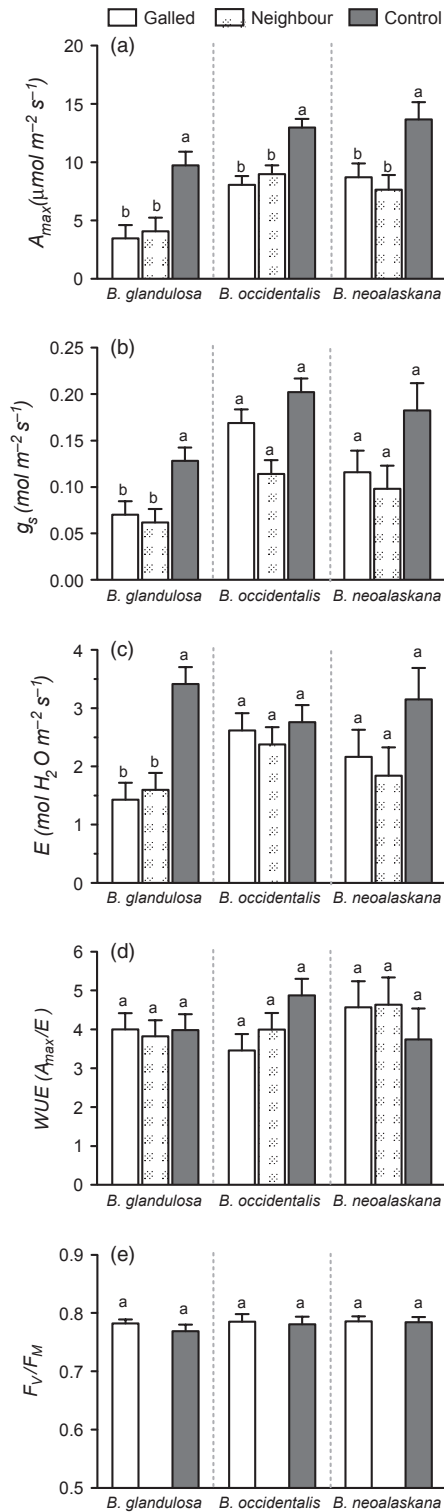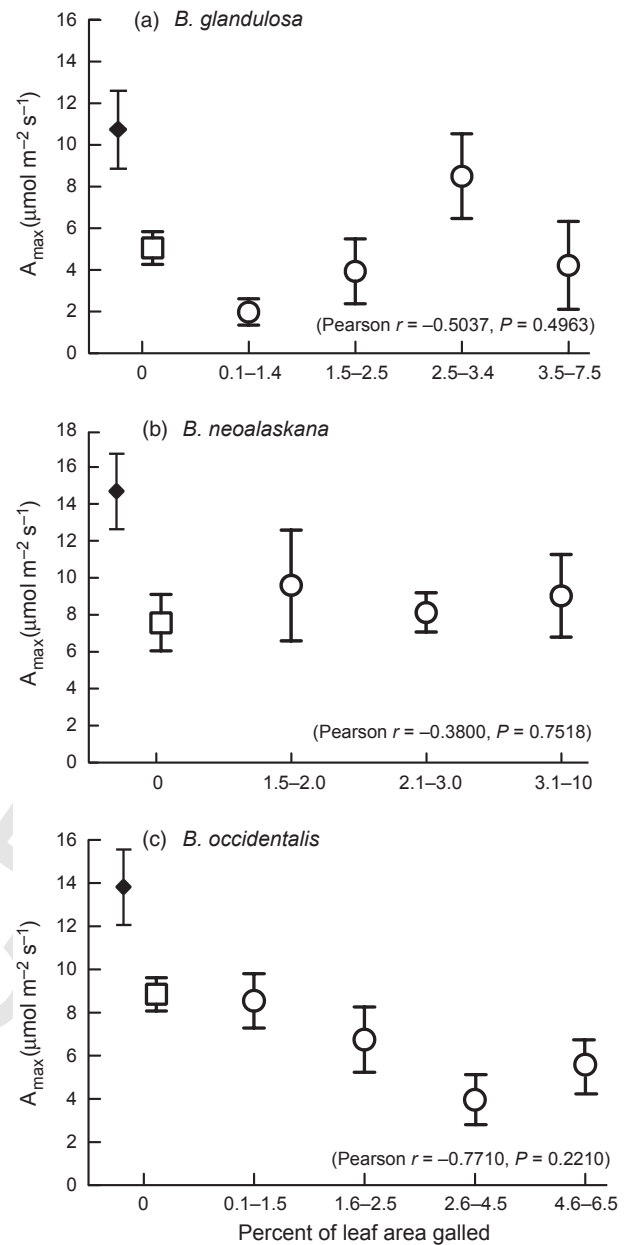
Fig. 4. Net photosynthetic capacity (A_{max}; a), stomatal conductance (g_s; b), evapotranspiration (E; c) instantaneous water use efficiency (WUE; d) and dark-adapted PS II quantum efficiency (F_V/F_M; e) measured in galled, neighbouring ungalled and 'control' leaves in three *Betula* species (*B. glandulosa*, *B. occidentalis* and *B. neoalaskana*) from Scotty Creek, Northwest Territories. For each species, different letters indicate a significant difference between groups at $P < 0.05$ (one-way ANOVA followed by Tukey's multiple comparison tests). Bars are mean + 1 SEM.

Fig. 5. Relationship between net photosynthetic capacity (A_{max}) and galling intensity (defined as per cent galled leaf area) in threes species of *Betula*: (a) *B. glandulosa*; (b) *B. neoalaskana*; and (c) *B. occidentalis*) – on permafrost plateaus in Scotty Creek, Northwest Territories. Open circles = binned A_{max} means (±1 SEM) from different galling intensities, open squares = A_{max} means (±1 SEM) of neighbouring plateaus leaves and filled diamonds = A_{max} means (±1 SEM) of ungalled leaves from gall-free plants.

Hartnett & Knapp 1993, 1996). We found no direct evidence to suggest that plants on permafrost plateaus experience greater water stress compared to other habitats (Table 1, Fig 3a); indeed there were no systematic differences in midday water stress between habitats or between galled and gall-free stems of *B. glandulosa* and all three habitats have reasonably high soil volumetric water content. However, other key differences may result in either an interaction between

herbivore-driven signalling and plant rooting conditions or other soil traits (e.g. beneficial rhizo-microbial communities; Pineda *et al.* 2010) that drive habitat-based responses to galling.

Data from Scotty Creek reveal that the soil thermal regimes of plateaus are distinct from the other two habitats. Specifically, on the plateaus, near-surface (0–10 cm) soil temperatures oscillate by 10 degrees (Fig 6a) diurnally during the growing season and reach much lower minimum temperatures during the winter months (as low as to −9 °C). This is in stark contrast to the relatively stable diurnal and less variable seasonal changes at the same shallow depths in the other two habitats (Figs 6b, c). At greater depths (30 and 50 cm), plant root systems on plateaus are subject to consistently lower soil temperatures (Fig 6a) during the growing season compared with those in bogs or fens (Fig 6b,c). Greater soil depths on the plateaus are closer to the permafrost zone, giving rise to a rapid thermal gradient through the shallow active (i.e. seasonally thaw) layer. Hence, plants (and in particular, root systems) on plateaus appear to be exposed to thermal stress both in terms of diurnal and seasonal temperature changes close to the surface, and lower soil temperatures at depths close to the frost table, implicating an interaction between mite-gall herbivory response and stress associated with a defining feature of the plateau habitat, permafrost.

Although the nature of such an interaction is at this point speculative, the photosynthetic down-regulation of neighbour leaves on plateaus suggests a root-derived, systemic defence response. The anticipated response is compensatory whereby photosynthetic rates of neighbouring tissues increase to support the 'sink' behaviour of nearby galled leaves or stems (e.g. Bagatto, Paquette & Shorthouse 1996; Fay, Hartnett & Knapp 1996; Patankar *et al.* 2013). Thus, it is possible that the response to galling is mediated by the growing condition of the root system; in this case, roots exposure to thermal stress associated with permafrost habitats. Root systems are often active participants in plant protection against aboveground herbivory (Erb *et al.* 2009) with leaf-derived signalling of herbivore attack resulting in induction of phytohormone production in the roots that signals a range of systemic defence responses (reviewed in Erb *et al.* 2009). In this case, the response to herbivore attack on the permafrost plateaus is stomatal closure and subsequent decreases in photosynthetic rates in both galled and neighbouring leaves (Fig 2), a response observed in all the three plateau species examined (Fig 4). Abscisic acid (hereafter ABA) controls stomatal aperture through its regulation of guard cell turgor pressure (Schroeder, Kwak & Allen 2001) implicating ABA biosynthesis in plant response to gall-mite herbivory. Previous studies have demonstrated that gall-mite herbivory increases the production of ethylene (Chakrabarti, Chakrabarti & Chakrabarti 2011; Samsone, Andersone & Ievinsh 2012) which serves as a direct defence response but also contributes to ABA biosynthesis (reviewed in Atkinson & Urwin 2012). Because this response is limited to the plateau habitat, it appears that the plant responses to gall-mite herbivory are interacting with additional stress factors specific to plateau environments. As described above, plateau soils are characterized during the growing season by hot surface soils and cool deeper soil layers. Associated with this thermal gradient is a gradient of increased soil moisture though all soil layers have reasonably high volumetric moisture (Table 1), a fact substantiated by the well-hydrated status of both galled and ungalled plants on plateaus (Fig 3a). Abscisic acid has been linked to tolerance of drought, waterlogging and cold temperature tolerance (Chandler & Robertson 1994; Mantyla, Lang & Palva 1995; Thomashow 1999). On plateaus, deeper soil layers are cold and nearly saturated (Fig. 6 and Table 1), which may be expected to lead to ABA production. Given that ABA-induced stomatal response requires sufficiently high tissue concentrations of ABA (Aasamaa *et al.* 2004) and that ABA forms a key player in convergent signalling pathways in multiple stress responses (Atkinson & Urwin 2012), ABA biosynthesis resulting from mite-gall herbivory may thus act in an additive way leading to the observed stomatal closure in
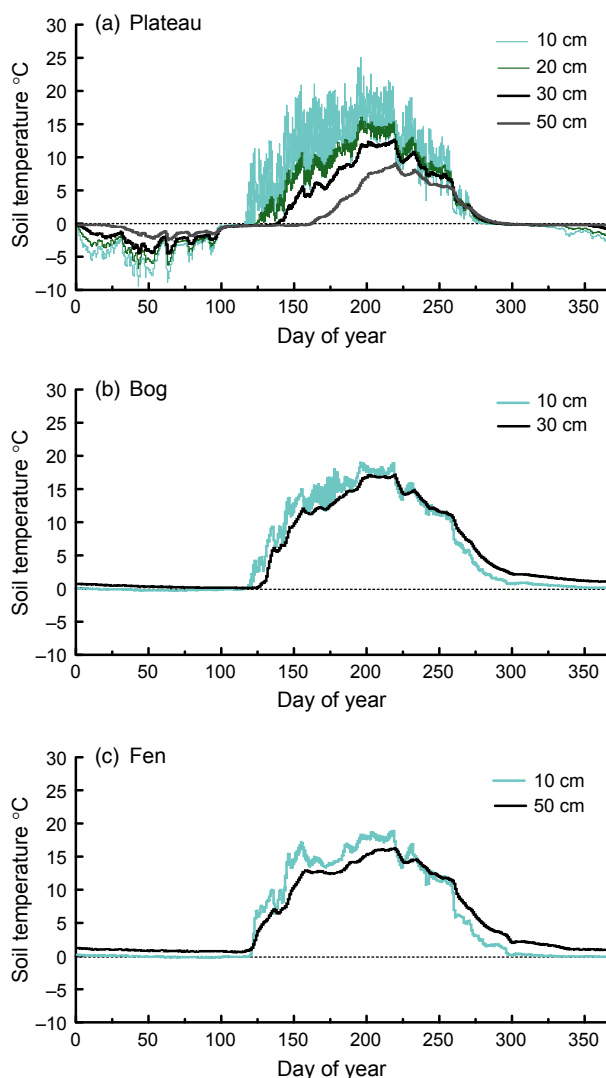
Fig. 6. Continuous (thirty-minute) soil temperature measurements in 2007 from (a) plateaus, (b) bogs and (c) fens from Scotty Creek, Northwest Territories. Bars represent temperature readings from different soil depths in the three habitats.

galled plants on plateaus (i.e. push marginally stressed plants to the stomatal closure threshold).

CROSS-SPECIES COMPARISONS OF GALL-MITE HERBIVORY RESPONSES

Galling had similar influences on gas-exchange processes in the two other species of *Betula*, with galled and neighbour *B. occidentalis* and *B. neoalaskana* leaves showing declines in net carbon uptake, thereby reinforcing patterns seen in *B. glandulosa* on plateaus. Overall, A_{max} declined by 37.8% (*B. occidentalis*) and 39.2% (*B. neoalaskana*) in galled leaves and by 30.8% (*B. occidentalis*) and 46.3% (*B. neoalaskana*) in neighbours. Stomatal conductance and transpiration were similarly lowered compared with control leaves in all three species, but only significantly so in galled and neighbour *B. glandulosa* leaves. The responses of all three *Betula* species on permafrost plateaus appear to be typical responses to galling herbivory (Welter 1989) involving the closure of leaf stomata upon attack, along with concomitant decreases in photosynthesis and stomatal conductance, and is likely to be triggered by host plant defence signalling (Kerchev *et al.* 2011). Dark-adapted quantum efficiency of Photosystem II did not differ between galled and control leaves in any of the species, again suggesting that the observed differences in gas-exchange rates might be exclusively due to changes in stomatal regulation, and that galling in these species does not result in depression of PS II efficiency seen in other gall–plant interactions (e.g. Aldea *et al.* 2006; Huang *et al.* 2011; Patankar *et al.* 2013). This further enforces the idea of systemic ABA-induced stomatal closure as opposed to physiological declines relating to photosystem activity. Remarkably, the observed decreases in carbon uptake rates on galled leaves in the three species occur even at low levels of galling intensity (i.e. not simply due to loss of photosynthetic area) as galls were estimated to occupy only a small percentage of total leaf areas (range: 0.1–10% leaf area; Fig 5), and photosynthetic rates remained low across the entire range of galling intensity. Similar decreases in carbon uptake in response to low mite-galling intensity has been observed in a temperate forest canopy (Patankar, Thomas & Smith 2011) lending support to the idea that even minimal levels of galling can significantly affect photosynthesis in deciduous host leaves. Measurements of diurnal stem water potential made on *B. occidentalis* and *B. neoalaskana* over a 3-day period revealed no marked differences in water stress between galled and gall-free plants, corroborating midday water potential measurements in *B. glandulosa,* which suggests that leaf-level galling has no impacts on whole plant water status despite reductions in stomatal conductance.

RESPONSES OF UNGALLED NEIGHBOURING LEAVES

Neighbouring leaves on galled *B. glandulosa* plateau plants show similarly large declines in photosynthetic activity (54.6%, 51.0% and 53.1% in *Amax*, *gs* and *E* respectively) and this response was similar across the three plateau species.

In bogs, neighbour leaves on *B. glandulosa* had consistently lower rates of *Amax, gs,* and *E* in comparison with control leaves, suggesting either potential stomatal down-regulation in response to robust uptake rates by galled leaves here or possible nutrient re-allocation away from neighbouring leaves and towards the gall (e.g. Bagatto, Paquette & Shorthouse 1996). However, there was no difference in physiological rates of galled and neighbour leaves (Fig. 2). The lack of compensatory response in neighbouring leaves in all three species and across habitats is in direct contrast to recent findings from the arctic tundra (Patankar *et al.* 2013), but is similar to the lack of compensatory responses in neighbouring leaves of *Prunus serotina* in response to mite galling (Larson 1998). One caveat that lends support to the findings in this study is that neighbour leaves were strictly adjacent to galled leaves; in the arctic *Salix* study, nearby ungalled leaves from the same stem as galled ones were treated as neighbours (strictly adjacent neighbours were uncommon).

Conclusions

This study demonstrates clear habitat-related differences in response to galling herbivory in commonly occurring deciduous species within a northern forest–wetland landscape. A recent preliminary survey in the above study area (D. Marshall, unpublished data) revealed that galling herbivory is ubiquitous in this system, and extends to the majority of deciduous (*Alnus*, *Salix* and *Betula*) and dominant coniferous species (*Picea*) found here. It is clear from this study that mite galling imparts significant stress on leaf-level physiological processes in a habitat-specific way. Hence on plateaus, galling may compound the stress that species are already facing as a consequence of permafrost thaw. There is also the possibility that this habitat-specific herbivory response may lead to positive feedbacks in this system. Given the ubiquitous nature of galling in this system and the demonstrated consistent physiological response across plateau species, one such potential feedback is enhanced rates of permafrost thaw. Gall-mite herbivory resulted in reductions of 50% or more in leaf-level transpiration. Transpiration impacts both the energy and water balance in any system via: (i) movement of water across the soil–plant–atmosphere continuum, (ii) evaporative cooling of foliar tissues. Warmer plant canopies will increase the down-welling of long-wave radiation and sensible heat, adding to the ground surface energy balance (Pomeroy *et al.* 2006) and potentially to the rate of ground thaw. For example, for each one-degree rise in leaf temperature, the long-wave exitance from the leaf surface increases by 5–6 W m^{-2} over the temperature range of 10–25 °C. The effect on the ground thaw of this additional energy flux is enhanced by the increased soil moisture below plants with diminished transpiration, since thermal conductivity increases with increasing soil wetness (Wright, Hayashi & Quinton 2009). Given the relatively small (~10–20%) differences in the annual surface energy budgets between permafrost and permafrost-free terrains at the study site (Quinton, Hayashi & Chasmer 2009), understanding such mechanisms is an important step towards

understanding the overall ecosystem response to climate warming–induced permafrost thaw. As such, they require further investigation to more confidently predict fully the potential impact of herbivory in rapidly warming and temperature-sensitive systems where herbivore pressure is expected to increase (Bale *et al.* 2002).

Acknowledgements

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Impacts of galling on deciduous hosts in a sub-arctic wetland-forest complex are variable, and are most pronounced in habitats and on individuals that are already exposed to abiotic stresses (such as cooler, more variable soil temperatures experienced by individuals on permafrost). Furthermore, galling can potentially contribute to localized thaw processes in these environments via **2** impacts on plant functioning.

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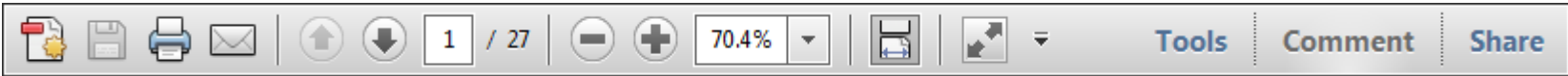
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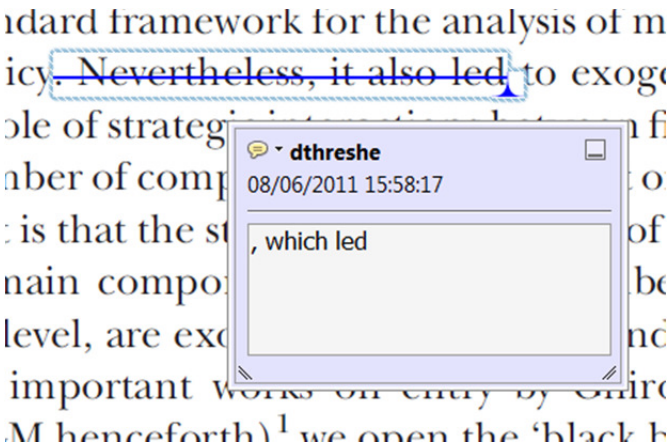
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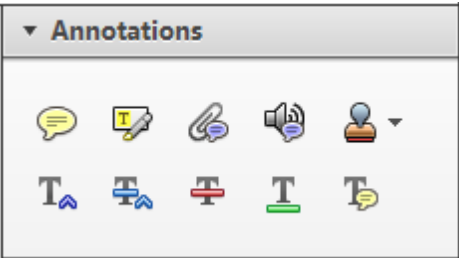
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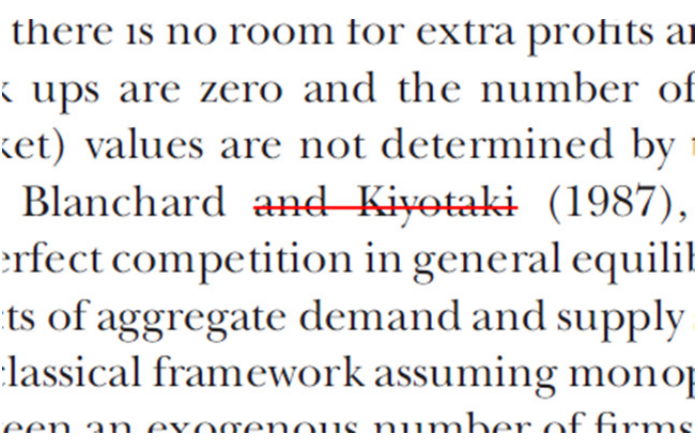idard framework for the analysis of m
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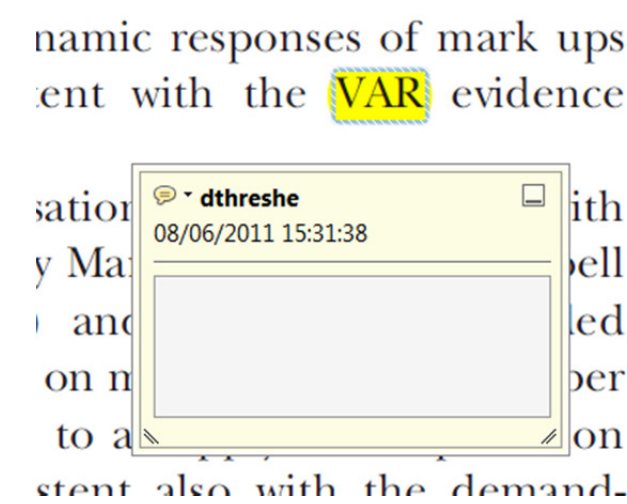## 3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

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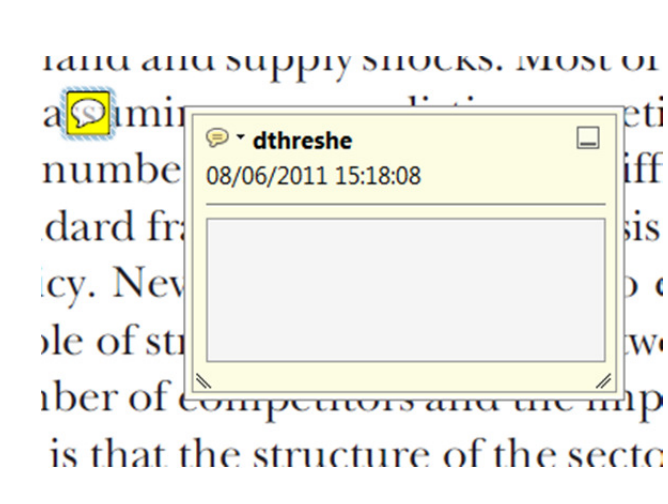- Type instruction on what should be changed regarding the text into the yellow box that appears.

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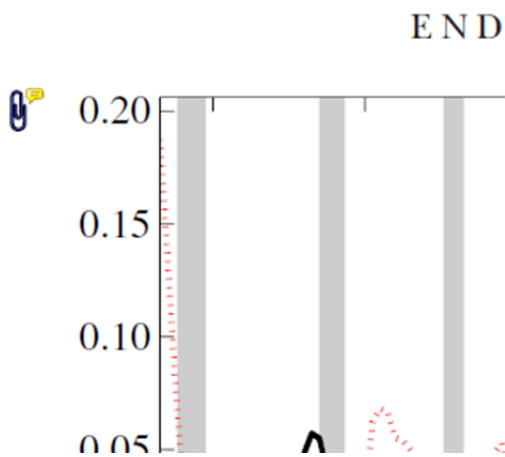
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).

or the business cycle, starting with the
. on perfect competition, constant ret
production. In this environment goods
extra **APPROVED** marke
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etermined by the model. The New-Key
otaki (1987), has introduced produc
general equilibrium models with nomin
ad and supply shocks. Most of this literat

7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

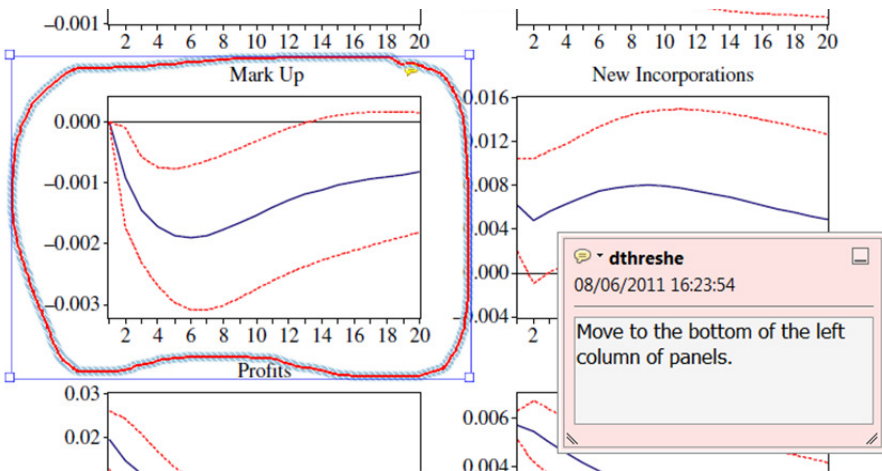Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.

> dthreshe
> 08/06/2011 16:23:54
> Move to the bottom of the left column of panels.

For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: