Title: Response of dark respiration to temperature in Eriophorum vaginatum from a 30 year old transplant experiment in Alaska

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Abstract

Background: In the Arctic region, temperature increases are expected to be greater under anticipated climate change than the global average. Understanding how dark respiration (R_d) of common Arctic plant species acclimates to changes in the environment is therefore important for predicting changes to the Arctic carbon balance.

Aims: To investigate the influence of genotype and growing environment on R_d, the temperature response (Q_{10}) of R_d, and foliar N (N_{leaf}) of the Arctic sedge *Eriophorum vaginatum.*

Method: We measured R_d, its Q_{10} and N_{leaf} of *E. vaginatum* populations that were reciprocally transplanted 30 years previously along a latitudinal transect of 370 km in northern Alaska.

Results: R_d and Q_{10} did not differ among populations (ecotypes) of *E. vaginatum,* but the local environment had a significant effect on both variables. R_d as well as N_{leaf} was higher in northern, colder sites, while Q_{10} was lower there.

Conclusions: R_d in the different populations of *E. vaginatum* is a very plastic trait and controlled by growing environment, as is N_{leaf}. The lower Q_{10} values in the northern sites were most likely a consequence of substrate inhibition of R_d at higher temperatures.

Keywords: Q_{10}, common garden experiment, leaf respiration, Eriophorum vaginatum, Arctic leaf nitrogen, reciprocal transplant

Introduction

Autotrophic respiration is a key component of the carbon budget of an ecosystem, contributing 30-65% of the total CO_2 released into the atmosphere (e.g. Janssen et al 2001; Luo et al. 2007). It is also widely acknowledged that understanding the long-term acclimation of autotrophic respiration is important in light of the anticipated global increase in temperature (e.g. Atkin and Tjoelker 2003; Luo et al. 2007; Atkin et al. 2008). Especially in the Arctic, temperature increases are expected to be greater than the global average (Solomon et al. 2007). Observations have already shown Arctic-wide warming trends since 1958 (0.11°C per decade (Kaufman et al. 2009), while Chapin et al. (2005) found that summer warming in arctic Alaska and western Canada accelerated to about 0.3° to 0.4°C per decade between 1961–2004. Currently, the Arctic acts as a modest carbon sink (McGuire et al 2009), but with changing temperatures it is important to understand how climate change can affect different components of carbon fluxes, such as autotrophic respiration.

Previous studies have shown that plant respiration acclimates to altered growth temperatures. For example, when measured at the same temperature, plants that have acclimated to lower growing temperatures had a higher foliar dark respiration (R_d) and a higher short-term temperature responsiveness of R_d compared with plants that were acclimated to higher growing temperatures (e.g. Strain and Chase 1966; Bolstad et al. 2003; Loveys et al. 2003; Bruhn et al. 2007; Campbell et al. 2007; Tjoelker et al. 2008). The short-term temperature responsiveness of R_d can be expressed by using Q_{10}, which represents the factor of change of R_d per 10 °C increase in temperature, usually measured between 10°C and 20°C. Atkin and Tjoelker (2003) described two different kinds of temperature acclimation of plant respiration: (1) altered temperature

sensitivity (i.e. a change in Q_{10}), and (2) a shift up or down in the overall temperature response curve (with no change in $Q_{10,}$ but with a change in the intercept of the response curve). The latter form of acclimation is likely to be more common in newly developed tissue, while the former occurs at a shorter time scale, in tissue that is already fully developed (e.g. Atkin et al. 2000; Ow et al. 2008). Long-term cold acclimation is also often accompanied by an increase in leaf nitrogen (N_{leaf}) (e.g. Tjoelker et al.1999; Lee et al. 2005; Tjoelker 2008), which is associated with increased investment in glycolytic and mitochondrial proteins. With more of these proteins, higher R_d rates at the same temperature are possible and consequently cold acclimation of R_d in the long-term (Atkin et al. 2005; Tjoelker et al. 2008).

One drawback of many studies of acclimation of respiration to temperature has been that the acclimation period at a lower or higher temperature has often encompassed periods ranging from one week or one growing season, which might not be a suitable time scale for studying effects of climate change. In this study, we present results from a 30-year-old reciprocal transplant experiment. In August 1980 and 1982, Shaver et al. (1986) established a latitudinal transect with six common gardens along the Dalton Highway in Alaska, in which whole tussocks of the sedge *Eriophorum vaginatum* L. were reciprocally transplanted over a distance of 370 km. Each of the gardens was located more than 200 m from the road in order to avoid artefacts of the road traffic (e.g., dust deposition). Each common garden included locally transplanted tussocks as well as tussocks that originated from the other garden sites. *E. vaginatum* is one of the most common and abundant species in northern Alaska (Britton 1966). It is a clonal species and individual tillers typically live less than 8 years (Fetcher and Shaver 1983; Mark et al. 1985), meaning that the entire biomass of the transplanted plants had been replaced at least 4 times before we

sampled them. The common gardens in the transect, which spans 3.30 degrees in latitude (~ 370 km), provided a unique opportunity to study the long-term acclimation of foliar respiration of plants from one location. In addition, they allowed us to determine whether different populations (ecotypes) of the same species had different physiological responses to changes in the growing environment. For the populations in the transect, differences in morphology and growth between ecotypes have been established previously (Shaver et al. 1986; Fetcher and Shaver 1990). Bennington et al. (2012) showed that tussocks that were retransplanted into their sites of origin had higher survival than tussocks from elsewhere on the transect, thus demonstrating home-site advantage. Likewise, we were interested whether differences in R_d rates between 'home and away' populations could be detected after 30 years of growth in the different sites. Additionally, we wanted to investigate if observed differences in rates or the temperature response of R_d were related to any changes in the values of N_{leaf}. The hypotheses tested in this study were: (1) There is no difference within a site amongst the transplanted tussocks (local vs. non-local origin) in their R_d rates, N_{leaf} , or Q_{10} values (i.e. there is no ecotypic variation). (2) Between sites (gardens), the R_d values at a standardised temperature are higher at the northern sites that have lower average temperatures. (3) The leaves from the colder, more northern sites have higher N_{leaf} values. (4) The Q_{10} values remain identical between sites, though the intercept of the temperature response curve differs).

Material and methods

Four of the six gardens described in Shaver et al. (1986) (No Name Creek, Coldfoot, Toolik Lake, and Sagwon) were visited between 16 and 23 July 2011 (Table 1). Two of these gardens are situated south of the Brooks Range (No Name Creek and Coldfoot), while the other two

(Toolik and Sagwon) are north of the Brooks Range. For 2011, the most northerly site (Sagwon) and the most southerly site (No Name creek) differed over 5 °C in average annual temperature (Table 1), and even more in the months leading up to the measurements (May-July 2011, Table 1). Additional details about the installation of the common gardens, environmental variation, and variation in growth and flowering can be found in Shaver et al. (1986), Fetcher and Shaver (1990), and Bennington et al. (2012). In this study, no individuals from the Coldfoot population were measured and because not all the transplanted tussocks had survived, a balanced design of measurements was not possible.

Temperature response curves for R_d were measured in situ between 9:00 am and 6:00 pm with portable photosynthesis equipment fitted with an expanded temperature control kit (Li-Cor 6400 and Li-Cor 6400-88, Li-Cor, Inc, Lincoln, USA). For each replicate, a selection of *E. vaginatum* leaves per tussock (3-9) was used. The mean ± SD leaf temperatures (T_{leaf}) ranged between 10 ± 2.0 °C and 25 ± 1.9 °C for each curve and R_d measurements were taken at intervals of ~ 2.5 °C. Each response curve took between 20 and 70 minutes, depending on how quickly the higher leaf chamber temperatures were reached. After the respiration measurements, the leaf samples were dried at 60 °C to a constant weight, ground and analysed for CHN with a Perkin-Elmer Series II 2400 CHNS/O Analyzer (LECO Corporation, U.S.A.). The response of R_d to T_{leaf} was fitted by regression using a modified Arrhenius equation (e.g. Lloyd and Taylor 1994; Griffin et al. 2002):

$$R_{dark} = a \cdot e^{bT_{leaf}}$$

where R_d is respiration rate, *a* and *b* are fitted parameters, respectively and T_{leaf} is leaf temperature. The Q_{10} values of the temperature response curve were then derived from:

$$Q_{10} = \frac{R_{dT+10}}{R_{dT}}$$

where R_{dT} and $R_{d(T + 10)}$ are respiration rates at the temperature of T_{leaf} and T_{leaf} +10. We chose this relatively simple equation because the range of temperatures at which we could measure R_d did not include the maximum temperature for leaf respiration, which is about 55 °C for *E. vaginatum* (O. O'Sullivan, pers. comm.). This made fitting the temperature response to other equations (for example, a polynomial equation) more difficult.

Statistical analyses were carried out in R with the *agricolae* package (R Development Core Team 2008). To analyse environmental (i.e. 'site' or 'garden') and genotype effects on R_d, Q_{10}, and N_{leaf}, we used an additive main effects multiplicative interaction (AMMI) model. Before the tests, the Q_{10} values were ln-transformed to obtain normally distributed data.

Results

The AMMI tests showed an effect of garden on R_d ($P < 0.04$), but no effect of population and no interaction between genotype and garden ($P < 0.29$ and 0.62, respectively). Hence, within the gardens, the *E. vaginatum* leaves from different populations did not differ in their R_d. Between gardens, leaves from Sagwon had the highest R_d at 10°C (Figure 1A). A similar result prevailed for Q_{10} values, as the AMMI tests showed an effect of the environment (i.e. garden) on Q_{10} ($P <$ 0.02), but no effect of population or interaction between population and garden ($P < 0.63$ and 0.89, respectively). Overall, the northern sites Sagwon and Toolik Lake had significantly lower values for Q_{10} than the two southern sites (Figure 1B). In contrast, both garden and garden * population significantly influenced the values for N_{leaf} ($P < 0.001$ and $P < 0.04$, respectively), with the northern gardens having larger values (Figure 1C), implying that N_{leaf} of the different populations responded differently to the transplantion.

Discussion

The 30-year-old reciprocal transplant study showed that R_d and Q_{10} in *E. vaginatum* populations were quite plastic. We found no significant effect of population within gardens on these traits, therefore our first hypothesis (H1) was supported, which is also agrees with the general finding of thermal acclimation of R_d in higher plants (e.g. Strain and Chase 1966; Bolstad et al. 2003; Zaragossa-Castells et al. 2007; Tjoelker et al. 2008; Rodriguez-Calcerdera et al. 2010). However, this result contrasts with the response of life history and morphological variables, such as tussock survival rate and tiller size from the same experiment. For these traits, Bennington et al. (2012) found home-site advantage (i.e. advantage for the population that originated in the common garden) in tussock survival rates, as well as greater plasticity in tiller size in *E. vaginatum* that originated from the southern sites. Therefore, the lack of difference between populations in plasticity in R_d and Q_{10} (which are measured at the tissue level) cannot be extrapolated to the functioning of *E. vaginatum* at the whole plant level, especially if some ecotypes produce fewer tillers in their 'away' environment. In other words, although the physiological parameters on a tissue scale do acclimate, there are some genetic based population differences (e.g. in survival rate, tiller length) that can limit the ability of plants to respond to a change in environment.

No difference in Q_{10} was expected between research sites (gardens), as Atkin and Tjoelker (2003) suggested that long-term thermal acclimation represents a shift up or down in the overall temperature response curve (no change in $Q_{10,}$ but with a change in the intercept of the response curve). However, the lower Q_{10} values in the colder, northern Sagwon site do not support our hypothesis (H4). Additionally, global differences in values of Q_{10} for R_d from Atkin and Tjoelker

(2003) show that colder, more northerly sites have higher, rather than lower Q_{10} values. In the context of these global patterns, it would be expected that a site, such as Sagwon would have higher, not lower, Q_{10} values when compared with the warmer, southern sites. It is possible that the latitudinal range included in this study (3.3 ° latitude) is not large enough to reflect patterns that are observed globally. In addition, the pattern of cold acclimation resulting in higher Q_{10} values, as described by Atkin and Tjoelker (2003), requires that in colder environments plants have a higher build-up of substrates (e.g. non-structural carbohydrates resulting from photosynthesis) due to a changed balance between R_d and foliar C uptake. The higher amount of substrate consequently allows for relatively higher R_d values with short-term warming, such as in a temperature response curve (Atkin and Tjoelker 2003). In our study, data on the non-structural carbohydrate content in the leaves is lacking. However, if this higher build-up of substrates did not occur in the colder, northern sites this could explain why the pattern described by Atkin and Tjoelker (2003) was not observed.

The increased values of N_{leaf} and R_d in the northern gardens (Figures 1A and 1C) supported our Hypotheses 2 and 3, and suggest an increased investment in the respiratory apparatus. Higher values for N_{leaf} at colder sites have been observed in boreal forest species (Tjoelker et al. 1999; Tjoelker et al. 2008) while increased protein levels and investment in mitochondrial volume have been found in cold-acclimated plants (Graham and Patterson 1983; Armstrong et al. 2006). Therefore, although the values of Q_{10} in the colder, northern sites do not suggest acclimation of R_d themselves, the higher values for $N_{leaf,}$ together with those for R_d give indirect support for a contrary conclusion. Higher values for R_d associated with lower values for Q_{10} at low temperatures have been observed in other studies. Xiong et al. (2000) found a greater temperature sensitivity of Antarctic species *Colobanthus quitensis* (Kunth) Bartl. and

Deschampsia antarctica E.Desv. when grown at higher (12 °C and 20 °C) rather than lower (7 °C) temperatures, but higher respiration in the cold-acclimated plants when measured at the same temperature. Larigauderie and Körner (1995), however, showed that thermal acclimation of a range of species can differ widely, both within genera, growth forms, and habitats. In sum, it might be hard to observe the effects of thermal acclimation of R_d in cold-acclimated environments through *in situ* measurements without factors, such as substrate limitation being considered.

Overall, under the anticipated warming of the Arctic, the *E. vaginatum* populations in Alaska will probably acclimate their R_d and the Q_{10} with higher temperatures. This implies that changes in this species' abundance or biomass following Arctic warming are a more important factor to consider when studying the effects of Arctic warming on the C balance of this ecosystem.

Conclusions

This study shows that R_d, Q_{10} and N_{leaf} are plastic traits in Alaskan populations of the species *E. vaginatum,* since the growing environments, rather than the genotypes explained most of the variation in these parameters. This thermal acclimation of R_d this species is probably facilitated through changes in protein levels and mitochondrial volume as indicated by changes in N_{leaf}. It has to be noted though that acclimation of R_d is a physiological response and that for the overall effects of Arctic warming on *E. vaginatum* other plant traits are important as well.

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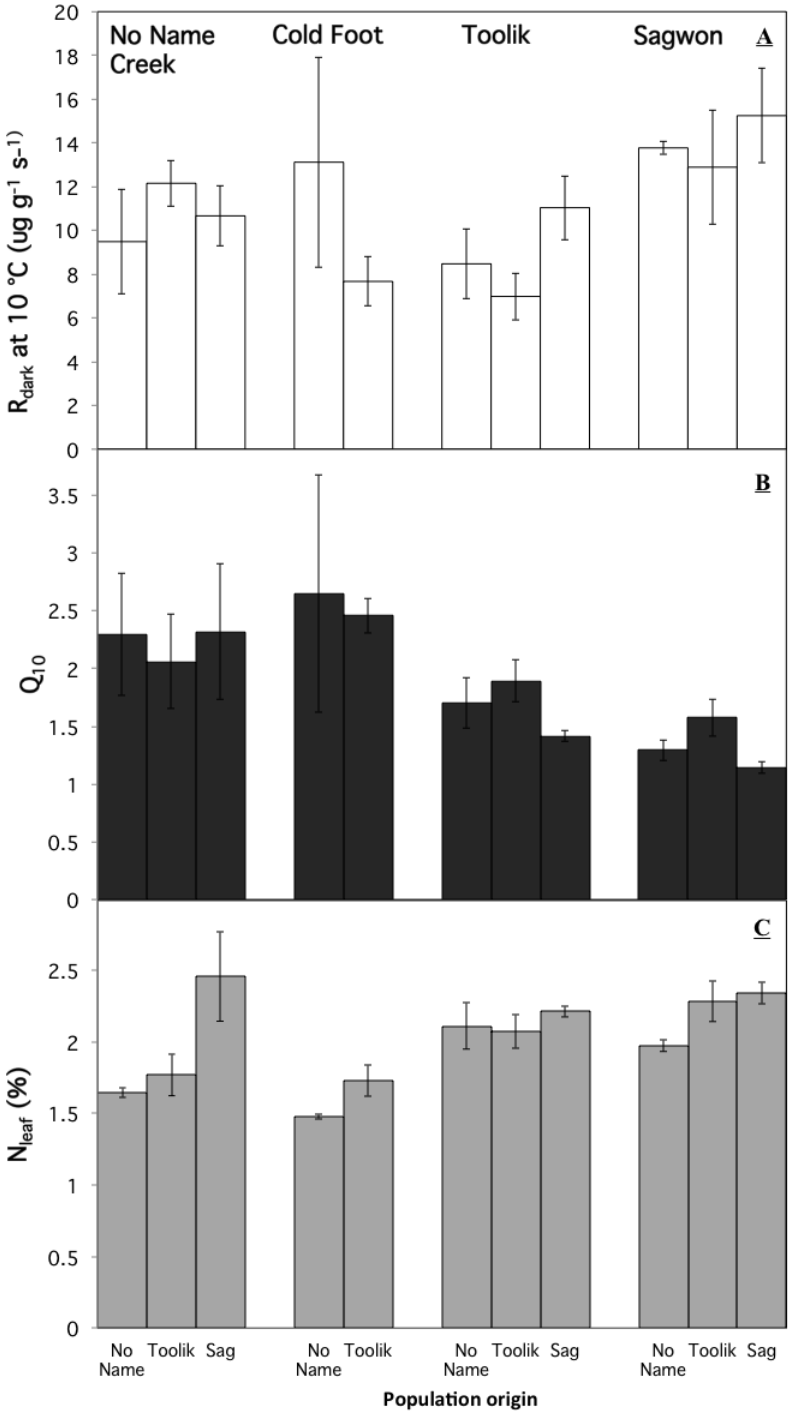
Figure legends

Figure 1. Average R_d at 10 °C (a), Q_{10} values of R_d (b) and average N_{leaf} (c) per population origin (denoted below) and common garden (denoted above) and their standard errors.


No Name Creek
Cold Foot
Toolik
Sagwon
A
B
C
R_{dark} at 10 °C (ug g^{-1} s^{-1})
Q_{10}
N_{leaf} (%)
No Name
Toolik
Sag
Population origin

302 **Figure 1.**

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