Linking ethylene to nitrogen-dependent leaf longevity of grass species in a temperate steppe

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Abstract

1. Leaf longevity plays an important role in linking ecophysiology of an individual leaf to the productivity of the whole plant. Leaf longevity is closely associated with nutrient status in soil such that high nitrogen (N) supply usually reduces leaf longevity in natural communities, though contrasting results are also seen. Ethylene, the classical plant hormone, is closely related to senescence, but whether ethylene is involved in N-dependent leaf longevity has not been investigated.

2. We conducted both pot and field experiments to examine the effects of N addition on leaf longevity, ethylene evolution and N content in leaves of two dominant species, *Agropyron cristatum* and *Stipa krylovii*, in a temperate typical steppe in northern China.

3. N addition increased leaf ethylene evolution and leaf N content, while it shortened leaf longevity. Inhibition of ethylene production with ethylene biosynthesis antagonist $CoCl_2$ reduced leaf ethylene production by 26.2% and prolonged leaf longevity by 2.7 days for *A. cristatum*. $CoCl_2$ addition alone increased leaf longevity by 3.0 days for *A. cristatum* (all cases, $P < 0.01$).

4. Leaf N content was highly negatively correlated with leaf longevity, and was strongly positively correlated with ethylene evolution. Both leaf N content and ethylene evolution reached the peak value at the same time and then began to decline.

5. *Synthesis*. These findings provide the first experimental evidence in support of the involvement of ethylene in N-induced decrease in leaf longevity. Our results of N-

induced reduction in leaf lifespan may have implications for terrestrial productivity response to N deposition.

Key words: ethylene evolution, Inner Mongolia, leaf longevity, leaf nitrogen, nitrogen addition, typical steppe

Introduction

Leaf longevity is an important plant functional trait that links ecophysiology of an individual leaf to productivity of the whole plant (Abeles, Morgan & Saltveit 1992; Kikuzawa & Ackerly 1999; Craine & Reich 2001). Many studies showed that leaf longevity and turnover in natural communities are intimately associated with leaf nitrogen (N) content such that high leaf N content is negatively correlated with leaf longevity (Reich, Walters & Ellsworth 1997; Shipley *et al.* 2006). Many observations have showed that N addition reduce leaf longevity (Craine, Wedin & Reich 2001; Ren *et al.* 2011). However, there have been inconsistent results at both the inter-species level and the intra-species level in terms of effects of N on leaf longevity. For instance, Aerts (1989) found that leaf lifespan is shortened by N addition in *Erica tetralix*, but not in *Calluna vulgaris*. Similar contrasting results are also found in four *Carex* species (Aerts & Decaluwe 1995), two grasses (*Lolium perenne* and *Festuca rubra*) (Erley, Rademacher & Kuhbauch 2002), and a eucalypt (*Eucalyptus grandis*) (Laclau *et al.* 2009). In an observational study, leaf life span of 32 grasses are found to be negatively correlated with specific leaf area, but not with nutrient availability (Ryser & Urbas 2000). Such inconsistency within and among studies indicate that the variability of leaf life span could be affected by an array of factors, and that it could also be a result of interaction of several factors at the site-, crown-, shoot- and leaf-levels (Schoettle & Fahey 1994). These variable results of the relationship between N and leaf longevity require mechanistic understanding how N level controls leaf longevity.

Leaf longevity is largely determined by leaf senescence, which is an essential constituent part of leaf development (Lin, Hsu & Kao 2002). Senescence is a complex and highly regulated process, in which macromolecules are degraded in an ordered progression and the components are subsequently transported from the senescing leaves to other growing parts of plants (Smart 1994; Roberts, Passeron & Barneix 2006). From perspectives of evolutionary ecology, old leaves should be replaced with new ones after they reach their maximum net carbon gain (Ackerly 1999). Field experiments have illustrated that N addition speeds up leaf senescence, suggesting that leaf longevity is partly controlled by N levels in soil (Craine, Wedin & Reich 2001; Ren *et al.* 2011). The dependence of leaf longevity on N addition has been accounted for by the balance between leaf carbon gain and cost. For example, high N supply favours leaf growth due to enhanced photosynthesis, thus speeding up leaf turnover and consequently shortening leaf longevity (Chabot & Hicks 1982; Kikuzawa 1991; Cordell *et al.* 2001). However, the mechanisms underlying the relationship between N levels and leaf longevity remain to be dissected.

Ethylene has long been recognized as an important plant hormone involved in leaf senescing and defoliation (Abeles, Morgan & Saltveit 1992). It has been well established that ethylene can be produced by all cells during plant development, and that the highest rates of ethylene evolution occurs in meristematic, stressed and ripening tissues (Abeles, Morgan & Saltveit 1992). Ethylene evolution responds to both biotic (van Loon, Geraats & Linthorst 2006) and abiotic (Morgan & Drew 1997) stress due to up-regulation of the ethylene biosynthesis, which has been recognized to

be a common phenomenon. The elevated ethylene may act as an important signal to modulate numerous biochemical and physiological processes. In addition, ethylene evolution has been reported to be closely associated with nutrient status in soils as ethylene evolution is stimulated when plants are exposed to deficiency in mineral nutrients such as phosphorus (Li *et al.* 2009), iron (Romera, Alcantara & De La Guardia 1999) and potassium (Shin & Schachtman 2004). Besides these essential elements, recent studies have also demonstrated that changes in root architecture in response to high external nitrate concentration are mediated by ethylene (Tian, Sun & Zhang 2009). Given that ethylene is closely associated with leaf senescence, and that ethylene evolution is positively dependent upon soil N availability (Feng & Barker 1993; Tian, Sun & Zhang 2009), it is likely that ethylene is involved in N-dependent changes in leaf longevity. However, no studies have been conducted to link ethylene production and N-dependent leaf longevity in the literature.

The typical steppe of Inner Mongolia in north China is a dominant vegetation type in the semiarid regions of Eurasia, and is sensitive to global climate change (Christensen *et al.* 2004; Kang *et al.* 2007). As N is one of the key limiting factors for plant growth in this area (Bai *et al.* 2010), predicted N deposition increase in this region (Galloway *et al.* 2004) would inevitably have impacts on growth and development of plants. Our recent work in the temperate steppe revealed that leaf longevity of two dominant species, *Agropyron cristatum* and *Stipa krylovii*, was significantly shortened in response to N addition (Ren *et al.* 2011). To elucidate the mechanisms underlying how N addition decreases leaf longevity, we evaluated the

role of ethylene in N-induced decrease in leaf longevity by both pot and field experiments. The main objectives of this study were to determine: (1) whether ethylene evolution is responsive to N addition; (2) whether leaf longevity is related to ethylene production, and (3) whether there is a link between ethylene evolution and leaf longevity under N addition.

Materials and methods

Study site and experimental design

Field and pot experiments were both conducted in a typical steppe at Duolun county (116°17′ E, 42°02′ N, elevation 1324 m a.s.l.), Inner Mongolia, China. The climate is temperate and semiarid with cold winters and warm summers (mean January and July temperatures of -17.5 °C and 18.9 °C, respectively). Mean annual temperature amounts to 2.1 °C and mean annual precipitation is about 380 mm. The major soil type in this area is chestnut soil with mean soil bulk density of 1.21 g cm^{-3} and pH of 7.29. The vegetation at the study site is dominated by two C_3 grasses (*Stipa krylovii* and *Agropyron cristatum*), and a forb (*Artemisia frigida*).

In the pot experiment, we used a complete random factorial design with two levels of two factors: N addition and cobalt chloride ($CoCl_2$, an inhibitor of ethylene evolution) addition. Each of the four treatments had five replicates and each replicate consisted of 10 pots. Each pot under N addition was added with 0.45 g N (equivalent to 10 g N m^{-2} yr^{-1}) in the form of urea applied on July 27 and August 11, 2010,

respectively. Treatment with the ethylene synthesis inhibitor $CoCl_2$ was conducted by treating plants at 10 μM on July 27 and was repeated approximately every four days. Equal amounts of solution without $CoCl_2$ were also added at the same time to the control pots. The volume of water added with or without $CoCl_2$ ranged from 100 ml to 500 ml depending on soil dryness. *A. cristatum* was used in the pot experiment. Seedlings, which were generally in identical growth status and similar size and colour from a natural steppe site close to our field experiment site, were transplanted to plastic pots on June 27. Each pot was planted with 6 seedlings. The plastic pots were 25 cm in diameter and 24 cm in height and filled with 5 kg *in situ* soil. The background values of total organic carbon, total N and total phosphorus in pot soil were 29.9, 2.6 and 0.6 g kg^{-1}, respectively. The transplanted seedlings were used for varying treatments after ~20 days of recovery following the transplanting. Before treatments with addition of N and $CoCl_2$, leaves from different pots were collected to obtain the background information, and subsequently ethylene evolution and N content in leaves were determined.

In the field experiment, we deployed a randomized block design, with five replicate blocks, each containing four 8 × 8 m^2 plots. Four plots in each block randomly received 0, 5, 10, 15 g N m^{-2} yr^{-1}, respectively, in the form of urea applied by two times, each half in early May and July. The two co-dominant species, *S. krylovii* and *A. cristatum* were selected for evaluating the effect of N addition on leaf longevity and ethylene evolution.

Measurements of leaf longevity

We followed the protocols used by Craine *et al.* (1999) and Craine and Reich (2001) to determine the average leaf longevity of selected species. Leaf census of *A. cristatum* was made every ten days from July to October in the pot experiment in 2010. Accordingly, leaf longevity was estimated from the cumulative leaf length via calculations following Craine *et al*. (1999) and Craine and Reich (2001). The protocols used for determination of leaf longevity have been described in detail in Ren *et al.* (2011). The leaf longevity calculation formula we used explicitly considered the leaves from various ages. For example, in the calculation equation, it included leaves with birthdate unknown, deadthdate unknown and leaves that were born and senesced within the experimental period (Craine *et al*. 1999). Therefore the leaf longevity calculations won't be affected by leaf age variations, if any.

Measurements of leaf ethylene evolution and nitrogen content

Leaves were removed from the individual plants under different treatments and placed in open vials (5 ml for *S. krylovii* in the field experiment and 10 ml for *A. cristatum* in both pot and field experiment) for 30 minutes, and then these vials were sealed with the gas-tight stopper. One millilitre gas was collected from the vials after 1 h under dark conditions and analyzed using a gas chromatograph with a photoionization detector and a packed Teflon column (GC-4400; East & West Analytical Company, Beijing, China). Thereafter, leaf samples were collected and dried at 65 °C to determine leaf N content.

We sampled leaves around once every four days from late-July to mid-September to determine leaf ethylene evolution and N content in the pot experiment. We

collected leaves weekly or biweekly from early-July to mid-September to measure the two parameters in the field experiment. Though both species are perennial, the old leaves dies in winter and new leaves grow in spring every year. All the leaves used in both pot and field experiments were from the current year growth. The leaves were generally from the third node from the top, which means that all leaves sampled should have relatively similar age. In addition, we also determined the N contents of senesced leaves when the whole individual plant wilted in mid-October.

Data analysis

Repeated measures analysis of variance (ANOVA) was used to examine the responses of leaf ethylene evolution and N content to N addition and $CoCl_2$ supply. ANOVA was further performed to test the effects of N and $CoCl_2$ addition on leaf longevity. Multiple comparison (Duncan-test) was also used to evaluate the differences among the experimental treatments. Pearson's correlation coefficients were employed to investigate correlations between leaf N content and longevity, between leaf N content and ethylene evolution, and between leaf ethylene evolution and longevity. All statistical analyses were performed using SAS software (version 8.2).

Results

$CoCl_2$ abolished N addition-dependent changes in leaf longevity

Leaf longevity of *A. cristatum* was significantly shortened from 61.5±0.5 to 57.4±0.4 days in response to N addition in the pot experiment (Fig. 1). Treatments with ethylene synthesis inhibitor $CoCl_2$ significantly prolonged leaf longevity of *A.*

cristatum from 61.5±0.5 to 64.5±0.3 days in the absence of N addition. The N addition-induced reduction in leaf longevity was significantly alleviated by $CoCl_2$ and leaf longevity increased from 57.4±0.3 to 59.9±0.3 days (Fig. 1).

N addition evoked ethylene evolution from leaves

To further evaluate the role of ethylene in the N addition-induced reduction in leaf longevity, the effect of N addition on ethylene evolution from the leaves of *A. cristatum* grown in pots was monitored. Ethylene evolution peaked at the 5^{th} day of N addition and it gradually declined thereafter. A similar transient increase in ethylene evolution was observed in response to another N addition (Fig. 2a). The overall ethylene evolution from leaves in N-added pots was significantly higher ($P < 0.001$) than that in control pots throughout the experimental period. Treatment with $CoCl_2$ led to a significant reduction in ethylene evolution from leaves in the absence of N addition (Fig. 2a). In addition, the N addition-induced ethylene evolution was markedly suppressed by $CoCl_2$ throughout the experimental period. For instance, the peak of ethylene evolution after N addition was reduced from 3.8±0.3 to 1.7±0.2 ppm g^{-1} FW h^{-1} by $CoCl_2$ (Table S1).

To examine whether the N addition-induced ethylene evolution can also occur in plants grown in the field, we investigated the response of ethylene evolution from leaves of *A. cristatum* and *S. krylovii* to N addition in a temperate steppe. Similar to pot experiments, a significant increase in ethylene evolution in response to N addition was observed in the field experiments for both *S. krylovii* (Fig. 2b) and *A. cristatum* (Fig. 2c). The magnitude of increase in ethylene evolution was dependent on amount

of N addition for *A. cristatum* ($P < 0.01$) but not for *S. krylovii* ($P > 0.05$). For instance, ethylene evolution from leaves of *A. cristatum* under N addition of 10 and 15 g N m^{-2} yr^{-1} was significantly higher than under addition of 5 g N m^{-2} yr^{-1} ($P < 0.01$, Fig. 2c, Table S2). The ethylene evolution from leaves of *S. krylovii* increased by 62.9%, 74.7% and 75.3% by application of 5, 10, 15 g N m^{-2} yr^{-1}, respectively (Fig. 2b, Table S2). But there were no significant differences in ethylene evolution among the three levels of N addition ($P > 0.05$).

N addition increased leaf nitrogen content

To examine whether the N addition-induced ethylene evolution is related to N content in leaves, N content in leaves of *A. cristatum* was determined in the pot experiment. As shown in Figure 3a, repeated measures ANOVA revealed that N addition led to a significant increase in leaf N content. $CoCl_2$ addition caused a small decrease (2.8%) in leaf N content compared with control when no N was added ($P < 0.01$), whereas the $CoCl_2$-induced reduction in leaf N content was abolished with N addition (Fig 3a inset, Table S3).

In the field experiment, leaf N content of *S. krylovii* increased proportionally with the N levels added such that leaf N content increased by 7.7, 15.6 and 16.4% in the N5, N10, and N15 treatments, respectively (Table S4). Moreover, leaf N content in the N5 treatment was significantly lower than the N10 and N15 treatments ($P < 0.001$, Fig. 3b, Table S4). A similar increase in leaf N content in response to N addition was also observed for *A. cristatum* in the field experiment ($P < 0.001$, Fig. 3c, Table S4).

Correlations among leaf longevity, ethylene evolution and leaf N content

Leaf longevity of *A. cristatum* grown in both pots and field was negatively correlated with leaf ethylene evolution ($P < 0.001$, Fig. 4a, e). There was also a significantly negative correlation between leaf longevity and ethylene evolution for *S. krylovii* grown in the field (no *S. krylovii* in pot experiments) ($P < 0.001$, Fig. 4c). Significant negative correlation was found between leaf longevity and leaf N content for *A. cristatum* in both pot and field experiments ($P < 0.001$, Fig. 4b, f). Leaf longevity was also highly negatively associated with leaf N content for *S. krylovii* grown in the field ($P < 0.01$, Fig. 4d). As a result of the negative correlations between leaf longevity and ethylene evolution, and between leaf longevity and leaf N content, the ethylene evolution from leaves of *A. cristatum* was positively correlated with leaf N content in the pot experiments ($P < 0.001$; Fig. 5a). A similar correlation between ethylene evolution and leaf N content was also observed in *A. cristatum* and *S. krylovii* in the field ($P < 0.001$, Fig. 5b, c).

Discussion

N addition and leaf longevity

Leaf longevity is related to a suite of leaf traits in morphological, ecological and physiological properties, including leaf N and P concentrations, leaf photosynthetic capacity, dark respiration rate, leaf mass per area, leaf specific area (Reich, Walters & Ellsworth 1997; Wright *et al.* 2004) although other factors, such as herbivory, root architecture, soil nutrient status, evolutionary history and biogeography are also important (Rogers & Clifford 1993; Diemer 1998; Warren & Adams 2004; Wagner *et*

al. 2008). Among these traits, leaf nutrient content in general, N content in particular, is of the most importance because leaf N is closely related to plant CO_2 assimilation and photosynthetic capacity. According to the carbon cost-benefit analysis, leaf life span is short when the initial photosynthetic capacity of the leaf is high (Kikuzawa 1991; Escudero, Mediavilla & Heilmeier 2008). Since a large proportion of the leaf N is used to produce photosynthetic enzymes, photosynthetic capacity is strongly correlated with leaf N concentration (Reich, Walters & Ellsworth 1997; Reich *et al.* 1999). Our results from both pot and field experiments showed that N addition increased leaf N content, decreased leaf longevity, and that there was significantly negative correlation between the leaf N content and longevity. Together with an earlier report showing consistent decrease in leaf longevity after N addition for five herbaceous species from different functional groups in this region (Ren *et al.* 2011), it indicates that N addition induced leaf longevity decrease is a common feature for species of this typical steppe. These findings are generally consistent with previous observational studies showing that leaf longevity is decreased with an increase in leaf N content for both herbaceous and woody plants (Reich, Walters & Ellsworth 1997; Reich *et al.* 1999; Craine, Wedin & Reich 2001; Wright *et al.* 2004; Tjoelker *et al.* 2005). Our results also corroborated results from several experiments demonstrating that increased N supply promotes photosynthetic activities and accelerates leaf turnover, thus leading to a reduction in leaf longevity (Shaver 1981; Aerts 1989; Balster & Marshall 2000; Craine & Reich 2001; Craine, Wedin & Reich 2001).

Ethylene and N addition-dependent changes in leaf longevity

It has been well established that ethylene is a key plant hormone associated with leaf senescence (Jackson & Osborne 1970; Grbic & Bleecker 1995). A burst of ethylene evolution from plants in response to different abiotic and biotic stresses has been widely observed. There are also reports demonstrating that ethylene evolution is closely associated with nutrient status (Morgan & Drew 1997). For instance, increased ethylene evolution has been observed with deficiency of potassium (Shin & Schachtman 2004), sulphate (Zuchi *et al.* 2009), phosphate (Li *et al.* 2009), and iron (Romera, Alcantara & De La Guardia 1999). In contrast to these mineral nutrients deficiency, an increase in ethylene evolution from plant roots has been reported when plants experienced high nitrate (Tian, Sun & Zhang 2009) and ammonia levels (Feng & Barker 1993). In this study, multiple lines of evidence support the regulatory role of ethylene in mediation of N addition-dependent leaf longevity. Firstly, N addition stimulated fast ethylene production and decreased leaf longevity from leaves of two dominant species of a temperate steppe in both pot and field experiments. Secondly, there were four treatments in this study: control, N addition, N+$CoCl_2$ addition, $CoCl_2$ addition. The leaf longevity decreased in the N addition treatment, but the decrease significantly alleviated when combining N addition with ethylene inhibitor, $CoCl_2$. Thirdly, when added with $CoCl_2$ alone, the leaf longevity significantly increased compared with control treatment. Therefore, the current experiment showed convincing evidence that ethylene plays an important role in regulating the N-dependent leaf longevity change. Direct application of ethylene in future experiments

would provide even stronger evidence in its role in controlling leaf longevity. The results also revealed that leaf ethylene evolution was positively correlated with leaf N contents, implying that leaf N content is probably a direct factor in modulating ethylene production.

The canopy leaf area/leaf amount could increase under N addition and thus increase self-shading, which could confound the role of ethylene evaluation on the N addition-induced decrease in leaf longevity. Leaf area and leaf amount data are not available for the current experiment. However, there are several lines of evidences indicate that leaf amount is not a confounding factor for our conclusion about ethylene effect on leaf longevity. Firstly, both control and $CoCl_2$ addition treatments were used and $CoCl_2$ addition alone unlikely change leaf amount and leaf area. The experimental results clearly showed that leaf longevity increased under the $CoCl_2$ addition treatment compared with control treatment, indicating the inhibited ethylene evolution plays an important role in leaf longevity. Secondly, because of the canopy structure of these grass leaves (e.g., loose canopy), shading is not a major limiting factor for plant growth. Thirdly, in one earlier report, we showed that N addition decreased leaf longevity but did not change aboveground biomass in one experimental year (2007) (Ren *et al.* 2011). Because aboveground biomass is typically positively related with leaf amount for grass species, the different leaf longevity and biomass results caused by N addition indicate that leaf amount and leaf longevity are not necessarily correlated.

In summary, we demonstrated that N addition induced marked increases in ethylene evolution from leaves of two dominant species grown in pots and field in a temperate steppe. Multiple lines of evidence showed that N addition-induced ethylene evolution was closely associated with the reduction in leaf longevity, providing the first experimental evidence in support of the involvement of ethylene in N-dependent decrease in leaf longevity. These findings may have important implications for predications of Eurasia temperate grassland responses to N deposition. N deposition has been predicted to increase globally (Vitousek *et al.* 1997) and the predicted rates are particularly high in the Eurasia area (Galloway *et al.* 2004). The increased N deposition is also predicted to cause substantial increase in net primary productivity (Galloway *et al.* 2008, Bobbink *et al.* 2010). However, these predictions rarely consider the negative effect of elevated N supply on plant leaf longevity. Combined with findings of consistent decrease in leaf longevity after N addition for five herbaceous species in this region (Ren *et al.* 2011), these results indicate that N deposition-induced reduction in leaf lifespan is a common feature in plants of the northern China temperate steppe. Reduction in leaf lifespan may partially offset the overall increase in productivity, at least for plants in the northern China temperate steppe.

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Figure legends

Fig. 1 Responses of leaf longevity to N and $CoCl_2$ addition in the pot experiment for *Agropyron cristatum*, a dominant species of the typical steppe. The dotted line shows the level of leaf longevity under control. Different letters above each column indicate statistically significant difference ($P < 0.05$).

Fig. 2 Effects of N and $CoCl_2$ addition on leaf ethylene evolution. Arrows show the timing of N addition either in the pot experiment (a) or in the field experiment (b and c). Ac, *Agropyron cristatum*; Sk, *Stipa krylovii*. In all cases, the statistical significance was $P < 0.001$ for both N addition and $CoCl_2$ supply in the pot experiment, and for N addition in the field experiment for the both studied species. Control, no N or $CoCl_2$ addition; N, N addition; $CoCl_2$, $CoCl_2$ supply; N + $CoCl_2$, combined addition of N and $CoCl_2$; N5, N10, N15, N added by 5, 10, 15 g m^{-2} yr^{-1}, respectively.

Fig. 3 Impacts of increased N addition and $CoCl_2$ supply on leaf N content. Arrows show the timing of N addition either in the pot experiment (a) or in the field experiment (b and c). Inset, average leaf N content of *Agropyron cristatum* with (N1) or without (N0) N addition and with (Co1) or without (Co0) $CoCl_2$ addition in the pot experiment. In all cases, the statistical significance was $P < 0.001$ for both N addition and $CoCl_2$ supply in the pot experiment, and for N addition in the field experiment for both species. Ac, *Agropyron cristatum*; Sk, *Stipa krylovii*. Control, no N or $CoCl_2$ addition; N, N addition; $CoCl_2$, $CoCl_2$ addition; N + $CoCl_2$, combined addition of N and $CoCl_2$; N5, N10, N15, N added by 5, 10, 15 g m^{-2} yr^{-1}, respectively.

Fig. 4 Relationships between leaf ethylene evolution, leaf longevity (left panel) and

leaf N content (right panel) in the pot experiment (a, b) and the field experiment (c through e). Control, no N or $CoCl_2$ addition; N, N addition; $CoCl_2$, $CoCl_2$ addition; N + $CoCl_2$, combined addition of N and $CoCl_2$. Ac, *Agropyron cristatum*; Sk, *Stipa krylovii*. a, $R^2 = 0.85$, $P < 0.001$; b, $R^2 = 0.76$, $P < 0.001$; c, $R^2 = 0.41$, $P < 0.05$; d, $R^2 = 0.85$, $P < 0.01$; e, $R^2 = 0.84$, $P < 0.001$; f, $R^2 = 0.97$, $P < 0.001$. For the field experiment, we made the correlation analysis by using leaf longevity data measured in 2008 and leaf ethylene evolution data measured in 2010, assuming that the leaf longevity of the two studied species in 2008 was comparable with that in 2010 since the two years had similar precipitation.

Fig. 5 Correlations between leaf N content and leaf ethylene evolution in both the pot experiment (a) and the field experiment (b, and c). Ac, *Agropyron cristatum*; Sk, *Stipa krylovii*. a, $R^2 = 0.52$, $P < 0.001$; b, $R^2 = 0.69$, $P < 0.001$; c, $R^2 = 0.80$, $P < 0.001$.

Fig. 1

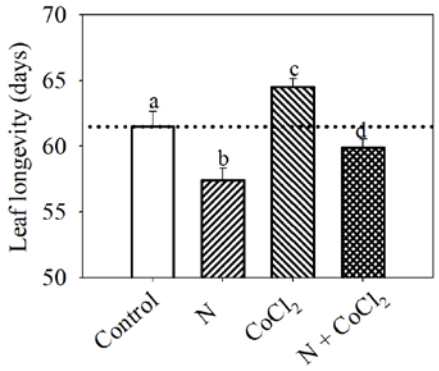

70
65
60
55
50
Leaf longevity (days)
a
b
c
d
Control
N
$CoCl_2$
$N + CoCl_2$

Fig. 2

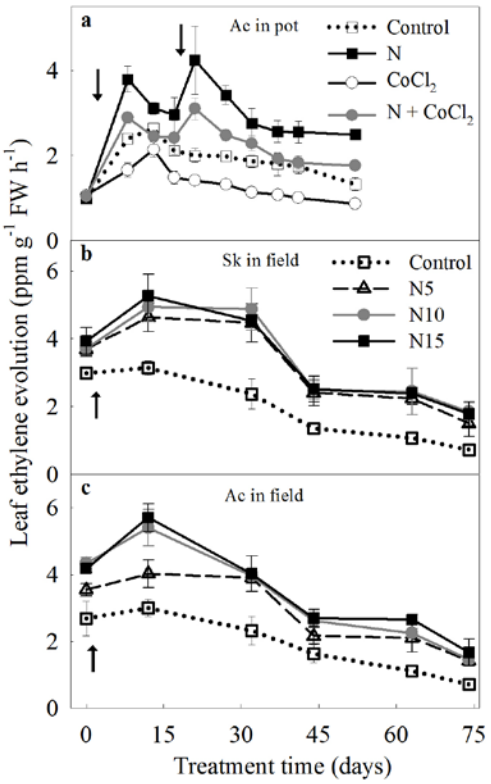

a
Ac in pot
Control
N
CoCl2
N + CoCl2
b
Sk in field
Control
N5
N10
N15
c
Ac in field
Leaf ethylene evolution (ppm g-1 FW h-1)
Treatment time (days)
0
15
30
45
60
75

Fig. 3

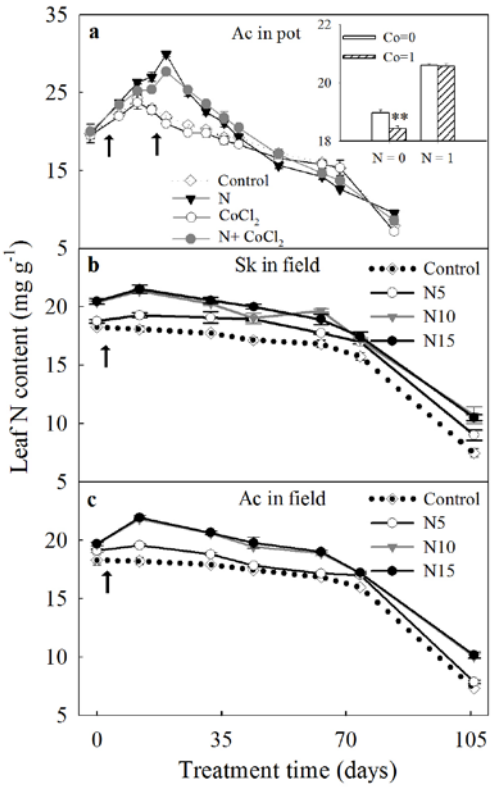

a
Ac in pot
Co=0
Co=1
**
N = 0
N = 1
Control
N
CoCl2
N+ CoCl2
b
Sk in field
Control
N5
N10
N15
c
Ac in field
Control
N5
N10
N15
Leaf N content (mg g-1)
Treatment time (days)
0
35
70
105

Fig. 4

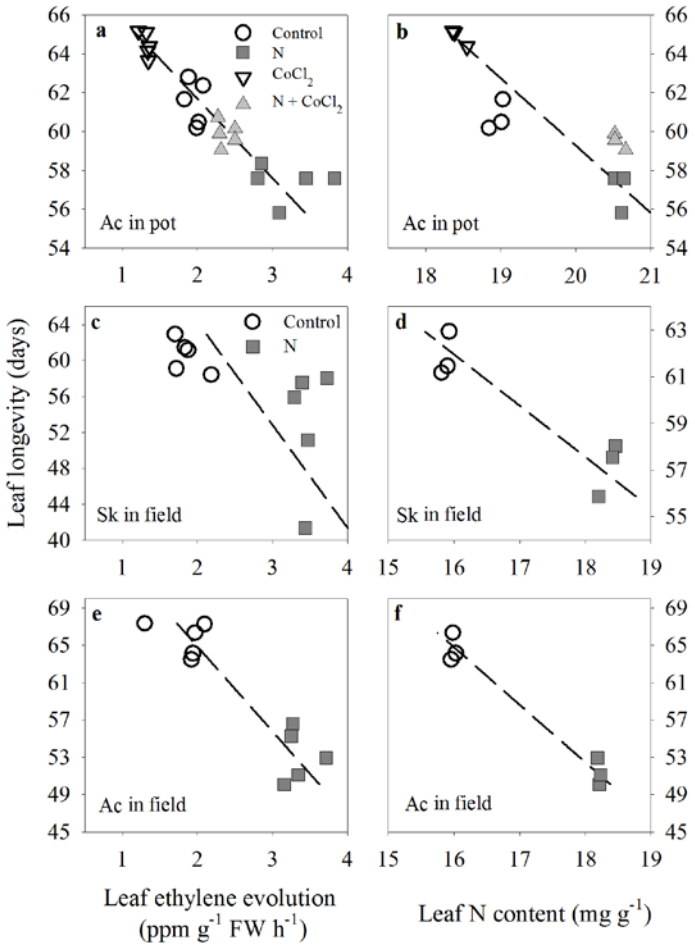
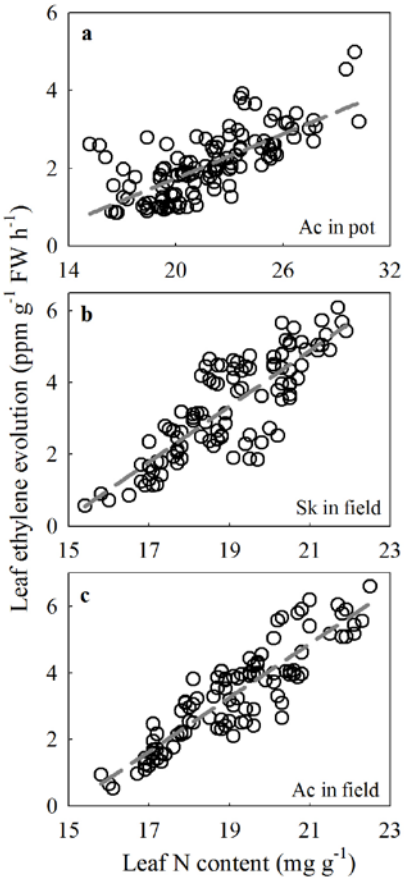

a
Control
N
CoCl2
N + CoCl2
Ac in pot
b
Ac in pot
c
Control
N
Sk in field
d
Sk in field
e
Ac in field
f
Ac in field
Leaf longevity (days)
Leaf ethylene evolution (ppm g-1 FW h-1)
Leaf N content (mg g-1)

Fig. 5


a
Ac in pot
b
Sk in field
c
Ac in field
Leaf ethylene evolution (ppm g-1 FW h-1)
Leaf N content (mg g-1)
0
2
4
6
14
20
26
32
15
17
19
21
23