

**LEAF- AND CELL-LEVEL CARBON CYCLING RESPONSES TO
A NITROGEN AND PHOSPHORUS GRADIENT IN TWO ARCTIC
TUNDRA SPECIES¹**

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- *Premise of the study:* Consequences of global climate change are detectable in the historically nitrogen- and phosphorus-limited Arctic tundra landscape and have implications for the terrestrial carbon cycle. Warmer temperatures and elevated soil nutrient availability associated with increased microbial activity may influence rates of photosynthesis and respiration.
- *Methods:* This study examined leaf-level gas exchange, cellular ultrastructure, and related leaf traits in two dominant tundra species, *Betula nana*, a woody shrub, and *Eriophorum vaginatum*, a tussock sedge, under a 3-yr-old treatment gradient of nitrogen (N) and phosphorus (P) fertilization in the North Slope of Alaska.
- *Key results:* Respiration increased with N and P addition—the highest rates corresponding to the highest concentrations of leaf N in both species. The inhibition of respiration by light (“Kok effect”) significantly reduced respiration rates in both species ($P < 0.001$), ranged from 12–63% (mean 34%), and generally decreased with fertilization for both species. However, in both species, observed rates of photosynthesis did not increase, and photosynthetic nitrogen use efficiency generally decreased under increasing fertilization. Chloroplast and mitochondrial size and density were highly sensitive to N and P fertilization ($P < 0.001$), though species interactions indicated divergent cellular organizational strategies.
- *Conclusions:* Results from this study demonstrate a species-specific decoupling of respiration and photosynthesis under N and P fertilization, implying an alteration of the carbon balance of the tundra ecosystem under future conditions.

Key words: Arctic tundra; carbon cycling; chloroplasts; light inhibition; mitochondria; nitrogen; phosphorus; photosynthesis; respiration.

The effects of increased nitrogen (N) and phosphorus (P) availability on carbon (C) cycling in Arctic tundra vegetation are important for the current understanding and future predictions of net C balance. The Arctic tundra is characterized by its vast C reservoirs (Gorham, 1991; Ping et al., 2008) and N and P limitation (Shaver and Chapin, 1986; Shaver et al., 1992; Hobbie et al., 2002), with C accumulating in the deep layer of organic matter sustained by permafrost (Dowding et al., 1981; Robinson and Wookey, 1997). Global change may alter this state (Chapin et al., 1995). Consequences of climate change on Arctic tundra ecosystems are evidenced through multiple cascading environmental and ecological effects, with implications for soil nutrient availability, changes in soil microbial mineralization, and C cycling. Increases in winter and summer temperatures (Serreze et al., 2000; Chapin et al., 2005; Anderson, 2010), lengthening of snow-free seasons (Stone et al.,

2002), and decreased albedo due to warming and land-surface changes (Chapin et al., 2005; Lundberg and Beringer, 2005) all affect biological processes underlying N and P resources for vegetation. Enabled by warmer conditions, the northward expansion of woody shrubs into tussock-dominated tundra continues to change plant community composition (Sturm et al., 2001; Tape et al., 2006). During winter months, the taller canopy height of shrubs allows for greater snowpack, creating an insulating layer that warms soil, and in turn can increase soil nutrient availability (Sturm et al., 2005). This, as well as observed differences in litter quality, decomposition rates, and soil microbial community structure, exhibit how ecosystem C, N, and P cycling can be altered under a shift to a more shrub-dominated landscape (Hobbie, 1996; Weintraub and Schimel, 2005; Wallenstein et al., 2007; Wookey et al., 2009). Further, warmer soil temperatures may increase rates of N and P mineralization (Nadelhoffer et al., 1991; Jonasson et al., 2004), adding to the available N pool. Also, despite the remoteness of the Arctic, N deposition from fossil fuel combustion is detectable (Woodin, 1997; Hodson et al., 2005) and is likely to increase in the future (Galloway et al., 2004). Though the direct anthropogenic source of N is relatively minimal, the combined impact of warming-mediated biological and environmental processes with deposition, will likely lead to a greater overall availability of limiting nutrients.

Long-term manipulation experiments of soil nutrient fertilization on Arctic tundra vegetation demonstrate the controlling

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influence of N and P on aboveground primary productivity (Shaver and Chapin, 1986; Johnson et al., 2000; Bret-Harte et al., 2004; Mack et al., 2004). Community responses to nutrient fertilization are well documented in tundra ecosystems (Shaver and Chapin, 1986; Shaver et al., 1998; Johnson et al., 2000; Hobbie et al., 2002; Bret-Harte et al., 2004; Mack et al., 2004). In tussock tundra, N+P fertilization can result in increased aboveground biomass, attributed to a compositional shift to a greater abundance of woody shrub species like *Betula nana* (Shaver and Chapin, 1986; Chapin et al., 1995; Bret-Harte et al., 2002; Mack et al., 2004). This shift suggests species and functional group differences that may be reflected in plant gas exchange. In wet sedge tundra, the community-level CO₂ fluxes of net ecosystem exchange (NEE), ecosystem respiration (ER), and gross ecosystem productivity (GEP) all increase under N+P fertilization, with the response to P being proportionally greater than that of N (Shaver et al., 1998). Similarly, when Johnson et al. (2000) considered the seasonal and diel impacts of CO₂ exchange in the same experimental plots, both ER and GEP were elevated significantly under N+P addition and exhibited the greatest divergence from the control plots during the peak season.

Species differences in carbon cycling can be especially important when considering the shifting ecological dynamics of the Arctic tundra. Because woody shrubs like *B. nana* expand both in individual size and geographic range, comparisons of leaf-level photosynthesis and respiration with the historically dominant tundra tussock *Eriophorum vaginatum* may strengthen predictions about the future tundra ecosystem function. Despite the large literature on tundra responses to nutrient addition, relatively few studies have examined leaf-level rates of gas exchange (Baddeley et al., 1994; Chapin and Shaver, 1996; Shaver et al., 1998; Bret-Harte et al., 2001). Of these, reports of N and P effects on photosynthetic and respiratory rates range from stimulation (Baddeley et al., 1994; Chapin and Shaver, 1996) to no change or inhibition (Shaver et al., 1998; Bret-Harte et al., 2001). Also, many studies on tundra vegetation use single-dose nutrient addition experiments, limiting the mechanistic understanding of these processes and their response to the environment. Single-dose fertilization experiments, when not compared with multilevel, multinutrient treatments, while informative, cannot capture possible threshold limits of physiological processes by N or P availability that may be observed at the scale of the community (Arens et al., 2008) or leaf (Baddeley et al., 1994). Previous work by Arens et al. (2008) in dwarf-shrub tundra reported a decline in GEP under higher N+P fertilization. Leaf measurements under graded nutrient availability may also reveal species interactions with N and P and more detailed seasonal effects (Baddeley et al., 1994). However, for both leaf- and community-level measurements of carbon exchange under fertilization, the duration of nutrient addition must be considered, and comparisons across different treatment time frames may be limiting in their interpretation.

To acquire a more accurate description of carbon cycling, we need measurements of respiration in the light (R_L) in addition to photosynthesis and dark respiration, as plants experience a near 24-h photoperiod during the growing season in the carbon-rich Arctic tundra, and neglecting this measurement can lead to inaccurate estimations of leaf carbon gain. Autotrophic respiration rates are inhibited by light—a phenomena known as the Kok effect—through multiple enzymatic pathways preceding or associated with the tricarboxylic acid cycle (Budde and Randall, 1990; Hill and Bryce, 1992; Hoefnagel et al., 1998;

Tovar-Méndez et al., 2003; Tcherkez et al., 2005; Rasmuson and Escobar, 2007). Previous studies show the degree of light inhibition can be sensitive to environmental factors such as ambient CO₂ concentration, growth irradiance, temperature, and water availability (Atkin et al., 2000, 2006; Hurry et al., 2005; Leegood et al., 1995; Wang et al., 2001; Shapiro et al., 2004; Pärnik et al., 2007; Tcherkez et al., 2008). However, given the multiple O₂ and CO₂ fluxes that occur concurrently in the light, respiration in the light cannot be measured directly. Methods have been developed to estimate R_L indirectly using stable isotopes (Weger et al., 1988; Turpin et al., 1990; Pinelli and Loreto, 2003), ¹⁴C (McCashin et al., 1988; Pärnik and Keerberg, 1995; Hurry et al., 1996), and gas exchange (Kok, 1948; Laisk, 1977; Sharp et al., 1984; Brooks and Farquhar, 1985; Villar et al., 1995; Peisker and Apel, 2001). Here, the Kok (1948) method, which calculates R_L based on the extrapolation of a low-light CO₂ assimilation curve is employed for this study based on its relative ease for measuring a high volume of replicates in the field.

In addition to gas exchange measurements, the characterization of pertinent leaf cellular organelles can be meaningful when addressing physiological responses to the environment. Mitochondria and chloroplasts mediate autotrophic carbon fluxes, and changes in their respective densities (based on direct counts using transmission electron microscopy) can indicate underlying adaptations, as evidenced in responses to elevated CO₂ (Robertson and Leech, 1995; Robertson et al., 1995; Griffin et al., 2001; Tissue et al., 2002; Wang et al., 2004; Gomez-Casanovas et al., 2007), canopy position (Tissue et al., 2002), and temperature (Armstrong et al., 2006a, b). Direct count measurements of mitochondria are rare and rarer still for field-grown species. Moreover, to our knowledge, no study has yet assessed how organelle abundance is affected by nutrient availability, either in Arctic or non-Arctic species. Such data could provide important insights into the underlying factors responsible for nutrient-dependent changes in metabolic rates in Arctic plants.

Given the C-rich, N- and P-limited setting of the Arctic tundra, as well as the potential sensitivity of tundra vegetation to warming-mediated environmental changes, a detailed characterization of leaf- and cell-level C-cycling processes in tundra vegetation will allow for greater understanding of the current and future carbon balance in this region. To examine the physiological, subcellular, and physical responses of Arctic plant leaves to elevated soil nutrient availability, we measured variables related to foliar C cycling in two dominant Arctic species of contrasting growth forms, *Betula nana* and *Eriophorum vaginatum*, under increasing levels of combined N and P addition. Using multilevel fertilized plots established in 2007 at the Arctic LTER in Toolik Lake, Alaska, we measured respiration in the light and the dark, photosynthesis, mitochondrial and chloroplast densities, and related leaf traits for samples collected in July 2009. These data were analyzed to test hypotheses about leaf- and cell-level C cycling: (1) that rates of photosynthesis and respiration would increase with combined nitrogen and phosphorus availability in a coupled manner; (2) the inhibition of respiration by light would decrease with increased fertilization, as suggested by Shapiro et al. (2004); and (3) the underlying cellular ultrastructure would correspond to leaf level fluxes. To our knowledge, this study is the first to characterize rates of leaf respiration in the light in Arctic plants, as well as the first to report on nutrient-mediated changes in organelle abundance.

MATERIALS AND METHODS

Site description and species—The study took place in July 2009 at the Arctic Long Term Ecological Research field station at Toolik Lake in the foothills region of the Brooks Range, North Slope, Alaska (68°38'N, 149°43'W, elevation 760 m a.s.l.). Soils in this area consist of 30–50 cm of a peaty organic layer and a silty mineral layer, with both atop permafrost. The average growing season of this region lasts approximately 10–12 wk, beginning in early to mid-June. Ambient light and temperature levels during the sampling period in late July fluctuated diurnally (Environmental Data Center Team, 2009; Fig. 1), and no large precipitation event occurred during this time.

Leaves were sampled from plots in a randomized block design (four replicate blocks) of seven fertilization treatments, created in 2007 by Gaius Shaver and colleagues on a large area of moist acidic tundra (MAT). Each year after snowmelt and prior to leaf-out, graded amounts of N and P, in the form of granular ammonium nitrate and triple superphosphate, were evenly distributed on the 5 × 20 m plots according to fertilization treatment. The grades of treatment increased proportionally: “CT” or “0N” (control, no fertilization), “0.5N” (0.5 g NH₄NO₃·m⁻² + 0.25 g P·m⁻²); “1N” (1 g NH₄NO₃·m⁻² + 0.5 g P·m⁻²); “2N” (2 g NH₄NO₃·m⁻² + 1 g P·m⁻²); “5N” (5 g NaNO₃·m⁻² + 2.5 g P·m⁻²); and “10N” (10 g NH₄NO₃·m⁻² + 5 g P·m⁻²). The experimental plots also included “nitrate-only” (5 g NaNO₃·m⁻² + 2.5 g P·m⁻²) and “ammonium-only” (5 g NH₄Cl·m⁻² + 2.5 g P·m⁻²) plots. For this study, leaves were sampled from the control (0N), 5N, and 10N plots only. It should be noted that these treatment levels, while denoted as 0N, 5N, and 10N, represent a combined dosage of nitrogen and phosphorus.

The species selected for this study represent the dominant tundra vegetation of the North Slope. *Eriophorum vaginatum* (cottongrass) is an evergreen tussock sedge, whose range spans the Arctic tundra and subboreal latitudes. *Betula nana* (dwarf birch) is a deciduous shrub, whose distribution covers Arctic, sub-Arctic, and alpine regions. Physiological measurements were made on fully illuminated leaves sampled from the top of the tundra canopy in July 2009. Care was taken to ensure a sampling of fully expanded leaves of a similar size and age for both species.

Foliar gas exchange—CO₂ assimilation rate as a response of light was measured under ambient (400 ppm) CO₂ concentration using a Li-Cor 6400xt infra-red gas analyzer (IRGA) (Li-Cor, Lincoln, Nebraska, USA). Four replicate measurements were made for each species under the three fertilization growth conditions corresponding to the four replicate blocks of the experimental plots. Cuvette block temperature was set to 20°C for all measurements to control for leaf temperature effects and is representative of temperatures experienced by leaves during the measurement period (Fig. 1). Relative humidity inside the leaf chamber was maintained between 30–60% during measurements, and leaf vapor pressure deficit average was 0.709 ± 0.075 (SE) across all treatments. Leaves were enclosed in the cuvette at high light conditions to acclimate prior to measurement. Measurements of CO₂ assimilation were recorded at photosynthetic photon flux density (PPFD) of 1500, 1200, 800, 400, 200, and 100 μmol·m⁻²·s⁻¹ and then by every 5 μmol·m⁻²·s⁻¹ decreasing to 0 μmol·m⁻²·s⁻¹. This range of light levels fully encompasses the light environment experienced by both species in their natural environment (Fig. 1). To estimate theoretically maximal light-saturated photosynthetic rate (A_{max}), we used curve-fitting software (Excel Solver version 12.0.4518, Microsoft, Redmond, Washington, USA) and a rectangular hyperbolic function. Dark respiration (R_D) was measured as the CO₂ efflux at zero irradiance. Photosynthetic rates at PPFD of 900 μmol·m⁻²·s⁻¹ (A_{900}) were also measured, as this light level is representative of the average light environment during sampling and measurement times (Fig. 1). For gas exchange, all leaves sampled were cut in the field, recut while

submerged under water, and measured inside in the laboratory. Previous experiments on these species showed no difference in rates of gas exchange and stomatal conductance between field-measured and laboratory-measured, cut leaves (K. L. Griffin, unpublished data). Multiple ($N \geq 10$) *E. vaginatum* leaves were laid across the base of the cuvette to cover the 6-cm² area. *Betula* leaves, which were smaller than the 6-cm² cuvette area, were measured for leaf area after IRGA measurement and assimilation values were calibrated to that area for calculations. Values of photosynthesis are expressed on an area and mass basis. Photosynthetic N-use efficiency (PNUE) is expressed as A_{max} per milligram N.

Quantifying respiration in the light using the Kok effect—Respiration in the light (R_L) was indirectly measured using the Kok (1948) method, which plots CO₂ assimilation as a response of light at low PPFD (<100 μmol·m⁻²·s⁻¹). Using the light curves described earlier, the low light points were analyzed using Excel Solver version 12.0.4518 to distinguish and fit two lines, one above and one below the “breakpoint” that occurs under low-light measurements (Shapiro et al., 2004). Where the line extrapolated from points above the breakpoint intersects the y-axis is considered R_L . The line fitted from points below the breakpoint intersects the y-axis at the R_D value, where PPFD = 0 μmol·m⁻²·s⁻¹. Respiration in the light was estimated after correcting for changes in internal CO₂ concentration according to Kirschbaum and Farquhar (1987), as described in Ayub et al. (2011). This correction accounted for changes in CO₂ concentration at the site of carboxylation that occur under decreasing light. The degree of inhibition of respiration by light is expressed as $I_{RL} = 1 - (R_L/R_D)$. These measurements were taken at a relative humidity of approximately 40–60%, and CO₂ concentration of 400 ppm. Potential diffusion in and out of the cuvette was accounted for, as was diffusion through the gasket, according to corrections presented in the LiCor 6400 Instructional Manual.

Measurement of O₂ consumption of leaves—Oxygen consumption by leaf tissue was measured using a Clark-type liquid phase oxygen electrode (Rank Brothers, Cambridge, UK; Hansatech Instruments, Norfolk, UK). Prior to measurement in the electrode, leaf tissue was sliced into small segments with a razor in 20 mmol/L MES buffer (pH = 6.0) and incubated in the dark for approximately 30 min. For uncoupled rates of oxygen consumption, 20 μmol/L carbonylcyanide *m*-chlorophenylhydrazone was added to the incubation and measurement buffers to achieve uncoupled (near maximal) rates of mitochondrial respiration. The electrode cuvette contained 2 mL of the same MES buffer for measurements, which were conducted at 20°C. The depletion of oxygen was recorded over ~5 min, and rates were calculated on a dry mass basis. Two replicates were measured for each species under each growth condition per replicate block for a total $N = 8$.

Organelle ultrastructure—Leaves were collected in late July and then fixed at 5°C in buffered glutaraldehyde (2% w/v in 0.05 mol/L potassium phosphate buffer), placed in sealed glass 20 mL vials, and transported in insulated containers from Alaska to Lamont-Doherty Earth Observatory in Palisades, New York, USA. Samples were postfixed in 2% (w/v) phosphate-buffered osmium tetroxide, dehydrated in graded acetone series, embedded in catalyzed epon (TAAB resin, Energy Beam Sciences, Agawan, Massachusetts, USA), and polymerized at 65°C. Ultrathin sections were obtained using a Porter-Blum MT-2 ultramicrotome, collected on uncoated copper grids, stained with Reynold's lead citrate, and examined with a Philips 201 transmission electron microscope (Eindhoven, Netherlands) operated at 60 kV accelerating voltage. The total number of mitochondria and chloroplasts were counted per cell section ($N = 45$ per species and treatment combination), and measured for size ($N = 30$, from a subset of cells measured for density), as described in Griffin et al. (2001). Each cell was measured for area, and mitochondrial density and chloroplast density (number per unit cell area, not including cell walls) were calculated.

Physical leaf traits and foliar nutrients—All leaf samples used for gas exchange measurements were also measured for leaf area using a flatbed scanner and computer with the WinRhizo program (Regent Instruments, Quebec City, Quebec, Canada). After the area was measured, leaf samples were dried in an oven at 60°C for a minimum of 2 d before mass was determined. After transport to New York, these samples were ground, weighed, and packaged for elemental analysis to determine [CHN] (2400 Series II, Perkin-Elmer, Boston, Massachusetts, USA). Remaining ground leaf samples were bulked by replicate block ($N = 4$) and sent to the North Carolina State University Environmental and Agricultural Testing Service (Raleigh, North Carolina, USA) for analysis by wet digestion to determine total phosphorus concentration.

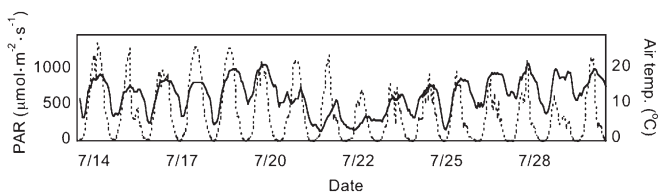


Fig. 1. Light (PAR; dashed line) and temperature (solid line) during the sampling period in July 2009, measured nearby within 100 m of the treatment plots at Toolik Lake, Alaska. PAR, photosynthetically active radiation.

Statistical design and analysis—Rates of photosynthesis and respiration, TEM organelle data, and leaf characteristics were analyzed using a two-way analysis of variance (ANOVA) with species and fertilization treatment as factors. Differences between fertilization levels were determined with post hoc Tukey's test. All data obtained from the experiment are expressed as means \pm SE. All analyses used the statistical programming software R version 2.11.1 (R Development Core Team, 2010).

RESULTS

Leaf nutrients and physical leaf traits—Leaf nitrogen concentration exhibited differences between species ($P < 0.001$, $F_{1,22} = 112.70$), treatments ($P < 0.001$, $F_{2,9} = 33.28$), and interaction effects were also found to be significant ($P < 0.005$, $F_{2,18} = 10.113$). In leaves of *E. vaginatum*, N concentration showed a general increasing trend from 0N and 5N to 10N, and in leaves of *B. nana*, this trend was significant ($P < 0.001$, Fig. 2). Highest

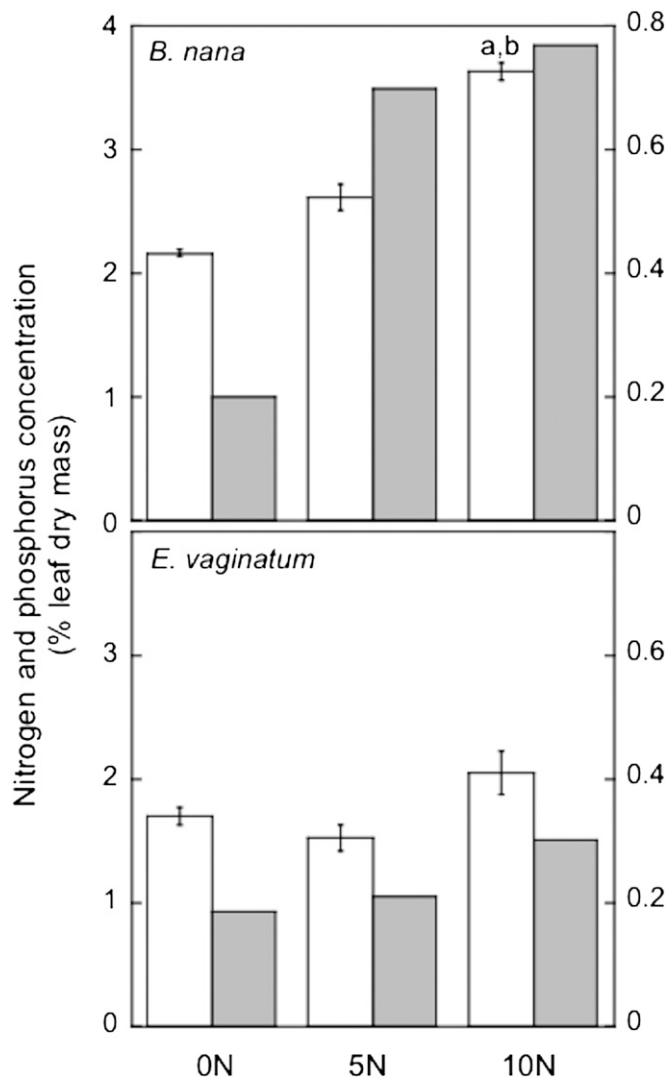


Fig. 2. Leaf nitrogen (unshaded, left axis) and phosphorus (shaded, right axis) concentration of *Betula nana* and *Eriophorum vaginatum* grown under the three fertilization treatments ($N = 4$). Values are means \pm SEM, with alphabetic notation signifying significance at $P < 0.05$ between the treatment levels. Limited sample size prohibited error to be calculated for phosphorus values.

N concentration for both species was measured in leaves grown under the 10N treatment (Fig. 2). Leaf carbon (C) concentration differed between species ($P < 0.05$, $F_{1,22} = 6.411$), with *B. nana* higher than *E. vaginatum* at all treatment levels, though there was no significant difference across treatments. Leaf C concentration was greatest at the 5N treatment level for both species. The ratio of C to N (C:N) differed between species ($P < 0.001$, $F_{1,22} = 88.80$), across treatments ($P < 0.001$, $F_{2,9} = 26.23$), and species–treatment interactions ($P < 0.05$, $F_{2,18} = 5.30$). *E. vaginatum* had higher C:N values at all treatment levels, with the greatest value observed at the 5N treatment level (30.33 ± 3.77). Values for C:N decreased in *B. nana* with increasing fertilization ($P < 0.01$). Leaf phosphorus values (Fig. 2) were limited statistically due to the small number of replicates, though the highest values for both species were recorded in leaves grown under the 10N treatment.

Specific leaf area (SLA), a measurement of leaf area per unit mass, differed at the species level ($P < 0.001$, $F_{1,22} = 29.74$), and from the 0N to 10N treatment in *E. vaginatum* ($P < 0.05$, Fig. 3). For both species, the highest mean SLA leaves were observed at the 10N treatment level, and values for *B. nana* were higher than *E. vaginatum* at each treatment level, though structural differences due to leaf type likely drive these differences. Leaf dry matter content (DMC) also differed between species ($P < 0.01$, $F_{1,22} = 8.67$) and treatment ($P < 0.05$, $F_{2,9} = 4.54$). Greater DMC was found in *B. nana*, and in both species, DMC decreased with increasing fertilization. Values of fresh mass per area (FMA) differed highly between species ($P < 0.0001$, $F_{1,22} = 749.74$), and nonsignificant fertilization and interaction effects were observed; *B. nana* increased slightly under nutrient addition, and FMA of *E. vaginatum* decreased by nearly 20% between the 0N to 10N levels.

Foliar gas exchange across a fertilization gradient—Area-based rates of maximal photosynthesis (A_{\max}) displayed no significant differences between the species or across the N and P treatment gradient, though mass-based rates of maximal photosynthesis were greater in *B. nana* ($P < 0.05$), and interaction effects were observed ($P < 0.01$; Table 1). The highest rates of carbon assimilation were observed under the 5N treatment of *B. nana* and under the 10N treatment of *E. vaginatum*, whereas the lowest rates were observed at 0N in *B. nana* and 5N for *E. vaginatum* (Table 2). Area- and mass-based rates of A_{900} differed in species–treatment interaction (Table 1), following the same trends as observed in A_{\max} (Table 2). A_{\max} displayed no clear, cross-taxa trend when expressed as a response to leaf N concentration (Fig. 4). PNUE exhibited treatment effects ($P < 0.01$) and was higher in *E. vaginatum* ($P < 0.001$). Greatest PNUE was observed under the 5N treatment in *B. nana*, and PNUE decreased under higher levels of fertilization in *E. vaginatum* (Fig. 5).

Area- and mass-based mitochondrial dark respiration rates were greater in *B. nana* than in *E. vaginatum* ($P < 0.001$ and 0.01, respectively), but displayed no treatment or interaction effects (Table 1). Highest area-based rates of R_D were observed under the 10N treatment level for both species (*B. nana*: $4.5 \pm 1.02 \mu\text{mol CO}_2\text{-m}^{-2}\text{-s}^{-1}$; *E. vaginatum*: $1.8 \pm 0.25 \mu\text{mol CO}_2\text{-m}^{-2}\text{-s}^{-1}$), and *B. nana* displayed a general increasing trend in rates of R_D across the fertilization gradient on an area and mass basis (Fig. 6A, 6C). When expressed on a mass basis, respiration decreased under increasing fertilization in *E. vaginatum* (Fig. 6D). A general cross-taxa increase in R_D was observed when expressed as a response to average leaf N concentration

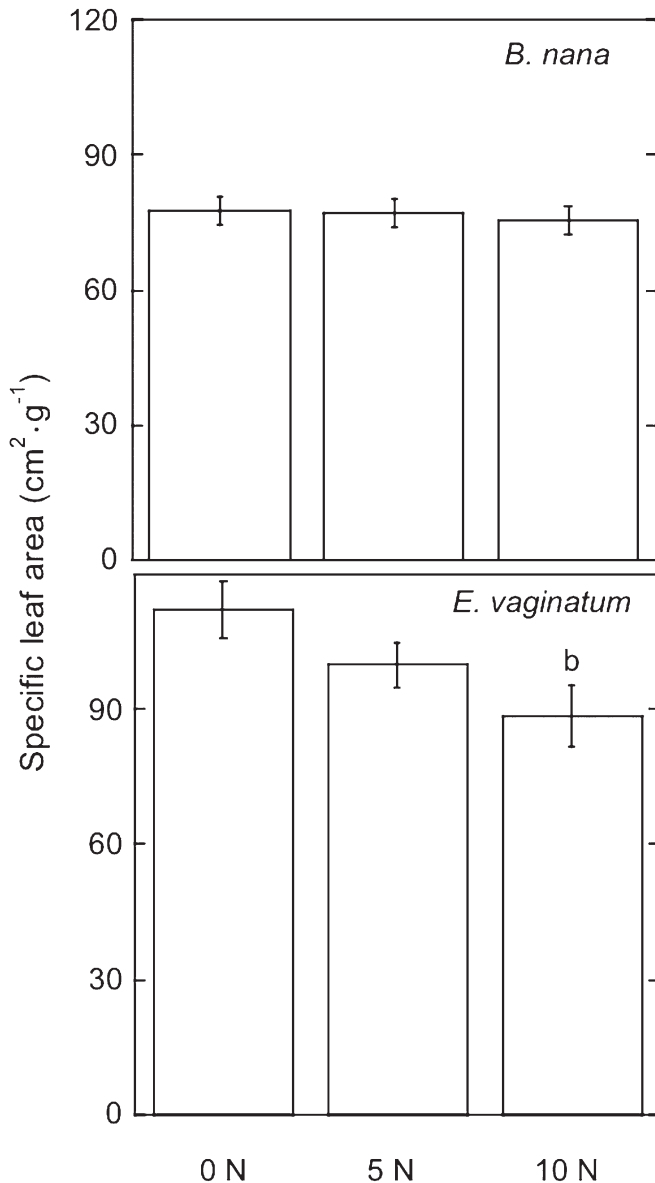


Fig. 3. Specific leaf area (SLA) of *Betula nana* and *Eriophorum vaginatum* under the three treatment levels ($N = 4$). Values are means \pm SEM and alphabetic notation signifying significance at $P < 0.05$ between the treatment levels.

(Fig. 4). Respiration in the light also differed by species ($P < 0.001$) with *B. nana* exhibiting higher rates at all fertilization levels (Fig. 6). Area- and mass-based rates of R_L did not differ significantly, but displayed an increasing trend in *B. nana* from the 0N to 10N level (Fig. 6A, 6C). Overall, average R_L increases with average leaf N concentration, though this relationship is stronger in *B. nana* (Fig. 4).

The net foliar carbon exchange, a ratio of photosynthetic to respiratory rates, was greater in *E. vaginatum* for both measurements of respiration ($P < 0.001$, Tables 1, 2). *E. vaginatum* had a higher net exchange than *B. nana* at the 0N and 10N levels for $A : R_D$, but not $A : R_L$. No difference was observed across fertilization treatments for either species for $A : R_D$ and $A : R_L$, though an interaction effect between species and treatment level was

TABLE 1. Two-way ANOVA results for photosynthetic and respiratory variables comparing *Betula nana* and *Eriophorum vaginatum* (species), nitrogen and phosphorus fertilization treatment levels (NP), and interaction effects (species \times NP).

Variable	Species (df = 1)		NP (df = 2)		Species \times NP (df = 2)	
	F- ratio	P	F- ratio	P	F- ratio	P
A_{\max} (area)	0.242	0.629	1.712	0.210	7.045	**
A_{\max} (mass)	5.544	*	1.018	0.382	7.970	**
A_{900} (area)	1.006	0.330	3.447	0.055	9.692	**
A_{900} (mass)	3.394	0.083	2.788	0.090	9.746	**
R_D (area)	28.471	***	1.826	0.191	1.928	0.176
R_D (mass)	13.317	**	0.495	0.618	3.206	0.066
R_L (area)	18.170	***	2.412	0.120	1.893	0.181
R_L (mass)	11.304	**	1.421	0.269	2.591	0.104
I_{RL}	2.160	0.160	1.192	0.328	0.186	0.832
$A : R_D$	46.112	***	0.418	0.665	3.880	**
$A : R_L$	16.954	***	0.246	0.785	0.607	0.556

Notes: Variables analyzed include area- and mass-based photosynthesis rates (A_{\max} , A_{900}), dark respiration (R_D), respiration in the light (R_L), degree of respiratory inhibition by light (I_{RL}), ratios of photosynthesis to respiration rates ($A : R_D$ and $A : R_L$), coupled respiration via oxygen consumption (R_C), and uncoupled respiration via oxygen consumption (R_{UC}). For A_{\max} , R_D , R_L , I_{RL} , $A : R_D$, and $A : R_L$, $N = 3-4$ for *B. nana* and $N = 4$ for *E. vaginatum*. Stars represent significance as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

observed in $A : R_D$ ($P < 0.01$, Table 1). The highest mean net exchange occurred in leaves grown in the 5N treatment plots for *B. nana* and in the 10N treatment plots for *E. vaginatum* (Table 2). Conversely, the lowest mean net exchange (considering both R_D and R_L) occurred in leaves from the highest fertilization treatment plots for *B. nana* and the 5N treatment plots for *E. vaginatum* (Table 2).

Measurements of coupled and uncoupled rates of dark respiration differed between species ($P < 0.01$, $F_{1,48} = 10.02$), but not in treatment level or interaction effects. The highest coupled rates of respiration were found in leaves grown in the 5N treatment plots for *B. nana* and the 10N treatment plot for *E. vaginatum* (Table 3), whereas the highest uncoupled rates were found in leaves grown under the 10N treatment in *B. nana* and the 0N treatment in *E. vaginatum*. The effect of the uncoupling agent CCCP varied in elevation in oxygen consumption rates (Table 3), with a mean stimulating effect of $13 \pm 4\%$ across both species and all treatments.

Light inhibition of respiration—Respiration was inhibited by light among all replicates ($P < 0.001$, $F_{1,21} = 359.15$), though species and treatment differences in the inhibition of respiration by light (I_{RL}) were not detected (Table 1). However, in both species, I_{RL} showed a decreasing trend with higher levels of N and P fertilization. The degree of the Kok effect ranged from 12–62% with a mean of $33.5 \pm 2.7\%$ across both species and all treatments. Though not significant, *B. nana* exhibited less light inhibition than *E. vaginatum* at all three treatment levels. Across both species, when expressed as a response to leaf N concentration, I_{RL} decreases with higher leaf N concentration (Fig. 4).

Organelle ultrastructural characteristics—Chloroplast and mitochondrial densities and sizes were influenced by fertilization treatments for both species (Fig. 7, Table 4). Chloroplast density exhibited treatment ($P < 0.001$), but not species or interaction effects (Table 4). Within species, *B. nana* chloroplast density increased from 0N and 5N to the 10N treatment level

TABLE 2. Mean (\pm SE) photosynthetic rates of *Betula nana* and *Eriophorum vaginatum* under fertilization treatments, including maximal photosynthesis (A_{\max}) and photosynthesis at photosynthetic photon flux density of 900 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (A_{900}), on an area and mass basis ($N = 4$). Values for the ratio of photosynthesis to respiration in the dark ($A : R_D$) and light ($A : R_L$) are also shown.

Treatment	$A_{\max(\text{area})}$ ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	$A_{900(\text{area})}$ ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	$A_{\max(\text{mass})}$ ($\text{nmol CO}_2\cdot\text{g}^{-1}\cdot\text{s}^{-1}$)	$A_{900(\text{mass})}$ ($\text{nmol CO}_2\cdot\text{g}^{-1}\cdot\text{s}^{-1}$)	$A : R_D$	$A : R_L$
<i>B. nana</i>						
0N	14.4 \pm 1.18	9.4 \pm 0.80	111.3 \pm 8.77	73.0 \pm 6.53	5.2 \pm 0.63	8.15 \pm 1.12
5N	24.5 \pm 3.26	17.6 \pm 2.30	189.9 \pm 28.40	136.9 \pm 20.12	7.1 \pm 0.62	10.2 \pm 1.68
10N	15.8 \pm 1.81	9.8 \pm 1.47	118.4 \pm 9.57	73.5 \pm 9.25	3.8 \pm 0.71	5.0 \pm 1.31
<i>E. vaginatum</i>						
0N	18.0 \pm 0.83	11.8 \pm 0.51	202.6 \pm 17.73	133.2 \pm 11.37	10.2 \pm 0.81	17.4 \pm 1.33
5N	15.2 \pm 0.93	10.1 \pm 0.33	150.8 \pm 4.61	100.6 \pm 3.22	9.4 \pm 0.90	16.7 \pm 4.77
10N	19.1 \pm 2.64	12.4 \pm 1.48	170.6 \pm 17.78	107.2 \pm 9.38	10.8 \pm 1.15	17.6 \pm 3.30

(both $P < 0.05$), and in *E. vaginatum* it increased from 0N to 10N ($P < 0.01$). Chloroplast size differed among effects based on species, treatment, and species-treatment interaction (all $P < 0.001$, Table 4). The largest mean chloroplast sizes were 3.3 μm^{-2} in *B. nana* under no fertilization treatment and 3.1 μm^{-2} in *E. vaginatum* under 10N treatment. *E. vaginatum* exhibited a general, though not significant, decreasing trend in chloroplast size from 0N to 10N, while *B. nana* increased from 0N to 5N and 5N to 10N. The mean total chloroplast area per cell increased 35% and 28% from 0N and 5N to 10N respectively, in *E. vaginatum*, whereas the same measure in *B. nana* increased over 500% from 0N to 10N (Fig. 8A, 8D).

Mitochondrial density differed by species ($P < 0.001$), fertilization treatment ($P < 0.001$), and the interaction effect ($P < 0.05$, Table 4). Mitochondrial density was greatest for both species at the 5N treatment level (*B. nana*: 0.18 mitochondria $\cdot\mu\text{m}^{-2}$ cell area; *E. vaginatum*: 0.11 mitochondria $\cdot\mu\text{m}^{-2}$ cell area), and higher densities were observed in *B. nana* at all treatment levels. Mitochondrial size was influenced by species, fertilization level, and the interaction ($P < 0.01$, 0.001, and 0.001, respectively, Table 4). Although no significant increase was observed between the 0N and 5N treatment in, mean mitochondrial size increased under 10N in *E. vaginatum* ($P < 0.001$). *Betula nana* did not show a similar increasing trend in mitochondrial size under fertilization. The largest mitochondria were observed in leaf sections sampled from the 10N treatment plot for *E. vaginatum* (0.24 μm^{-2}), and in leaf sections from the control plots for *B. nana* (0.21 μm^{-2}). The mean total mitochondrial area per cell increased 267% in *E. vaginatum*, and 27% in *B. nana* as fertilization increased from 0N to 10N (Fig. 8B, 8E). The ratio of total average chloroplast area to total average mitochondria area per cell was greatest under the 10N treatment in *B. nana* (Fig. 8C) and under the control treatment in *E. vaginatum* (Fig. 8F).

DISCUSSION

This study presents data on the responses of photosynthesis and respiration under increasing fertilization levels in two dominant Arctic tundra species. We also report, for the first time, measurements of the light inhibition of respiration in these species, and the effects of increased N and P availability on this effect, as well as on mitochondria and chloroplast density and abundance. We evaluated these leaf-level and cellular fine structure responses to increased N and P fertilization in arctic tundra plants with the hypothesis that gas exchange rates, related chloroplast and mitochondria size and density per unit cell area, and leaf traits will respond in a coupled manner.

Decoupled rates of gas exchange under nutrient addition—

The photosynthetic rates (A_{\max} and A_{900}) of both *B. nana* and *E. vaginatum* diverged from a direct trend in response to N and P fertilization and did not correlate with leaf N concentration. Previous work by Chapin and Shaver (1996), using the same fertilization treatment as the 10N level for this study for approximately the same duration (~ 3 yr), found a significant increase in leaf-level photosynthesis under nutrient addition for *B. nana* and *E. vaginatum* that was also observed in two other tundra species. In contrast, Bret-Harte et al. (2001) observed a decrease in leaf-level photosynthetic rate in *B. nana* grown for 8 yr under the same fertilization application as Chapin and Shaver (1996), suggesting either potential long-term acclimation or diminishing impact of increased soil nutrient availability. These examples suggest an influence of treatment duration on gas exchange measurements in these species. A similar response was documented in another tussock sedge, *Eriophorum angustifolium*, under the same treatment application for ~ 5 yr—photosynthesis decreased, while rates of respiration were unaffected by fertilization (Shaver et al., 1998). Another mechanistic explanation for this response may be N saturation, where increased leaf N is not accompanied by a coupled increase in photosynthetic capacity (Aber et al., 1989; Bauer et al., 2004), and this phenomenon has been documented in tundra vegetation at the community scale (Arens et al., 2008). Further, both species in this study exhibit the lowest PNUE at the highest fertilization treatment (Fig. 5) and highest leaf N, suggesting the allocation of N to nonphotosynthetic proteins, such as nitrate storage or cell wall material (Takashima et al., 2004). This trend is underscored by the tandem decrease in SLA under the 10N treatment in the evergreen *E. vaginatum* (Fig. 3). Seasonal and developmental timing, which can produce interactive effects on gas exchange in tundra vegetation under nutrient addition, may also explain differences in measured responses across studies (Atkin and Cummins, 1994; Baddeley et al., 1994; Illeris and Jonasson, 1999). Environmental heterogeneity across measurement years (Boelman et al., 2005; Arens et al., 2008) may further complicate cross-study comparisons of nutrient influences on carbon cycling. The decoupling of carbon assimilation with leaf nitrogen and fertilization treatment and the pronounced species differences emphasize the need to evaluate the broader dynamics of leaf carbon balance when considering the response of Arctic ecosystems to environmental change.

Respiration generally responded more directly to fertilization in *B. nana* than in *E. vaginatum* for both R_L and R_D , underscored by differences in leaf concentrations of N and P (Figs. 2, 4). These differences may be explained by functional differences between the species, such as (1) their source of N acquisition—*B. nana* forms ectomycorrhizal associations, and *E. vaginatum*

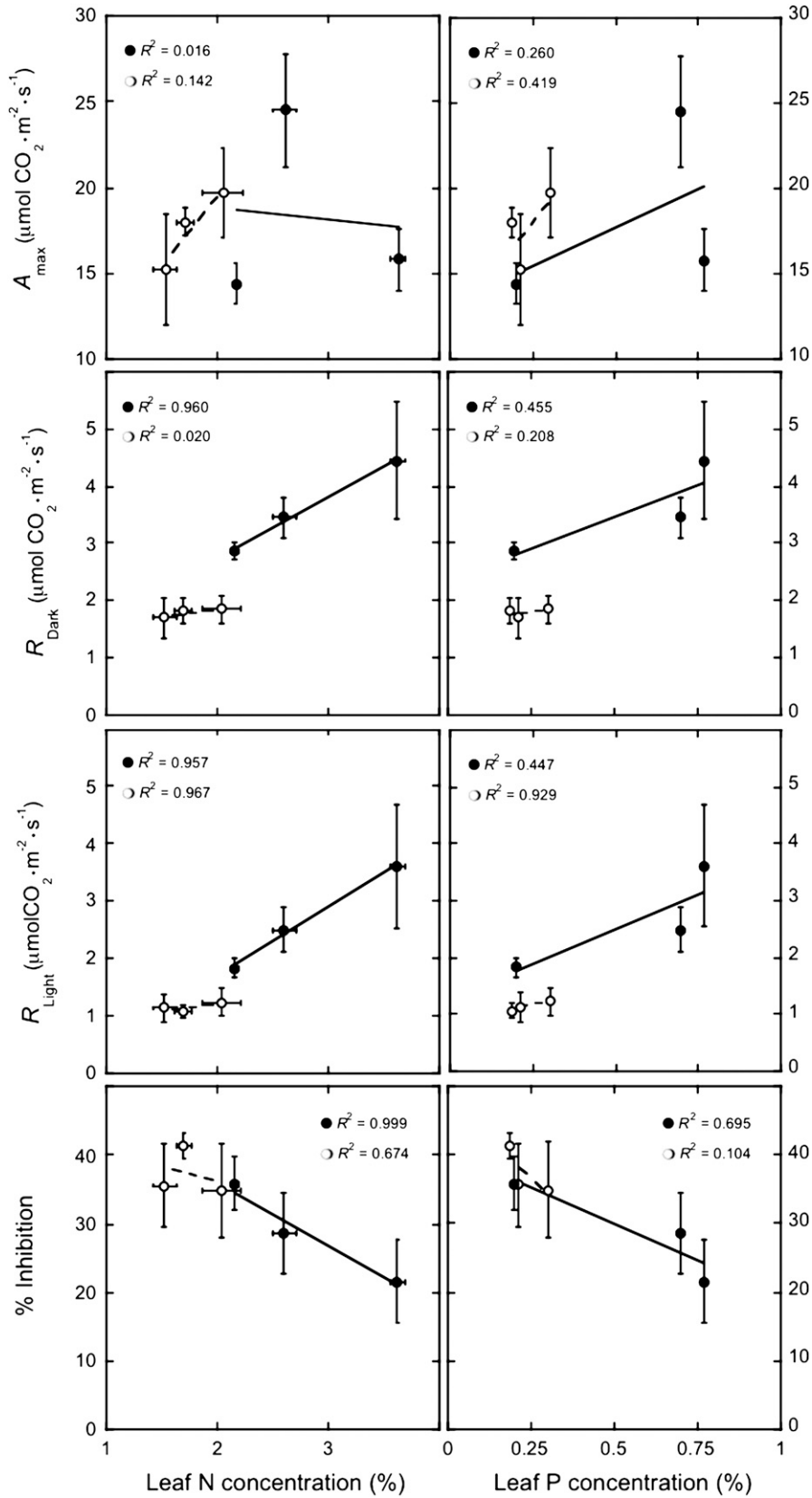


Fig. 4. Rates of foliar CO₂ exchange, including maximum photosynthesis (A_{max}), dark respiration (R_{Dark}), respiration in the light (R_{Light}), and degree of inhibition of respiration by light (% Inhibition) in *Betula nana* (filled) and *Eriophorum vaginatum* (unfilled) as a response of leaf N and P concentrations ($N = 4$). Values are means \pm SEM and adjusted R^2 .

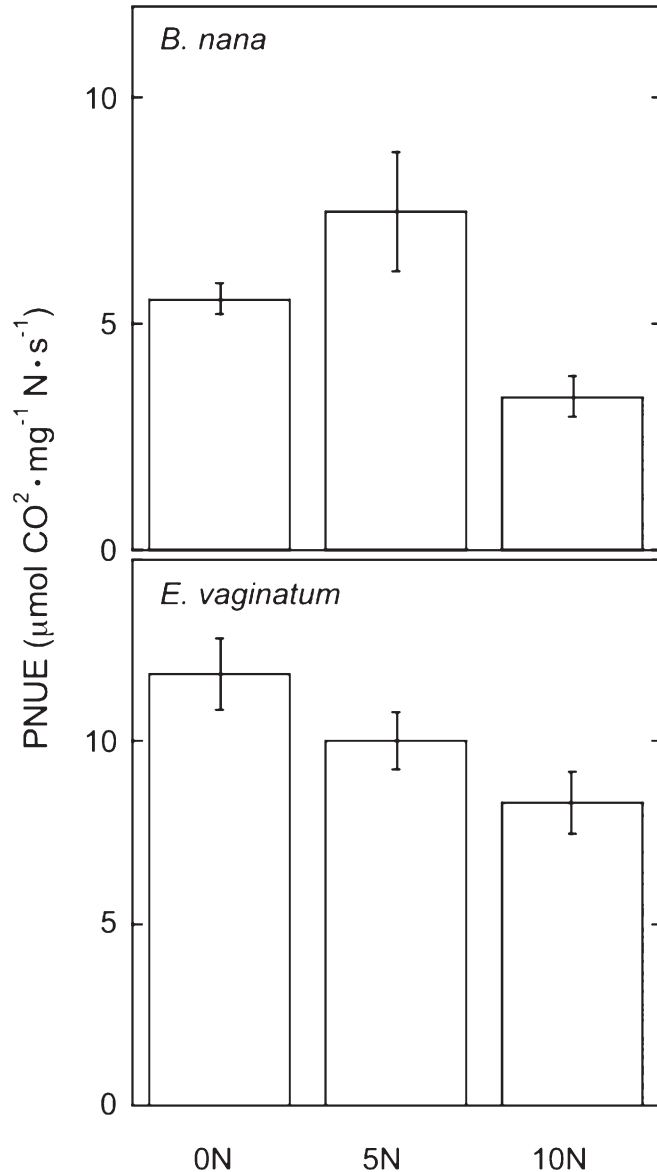


Fig. 5. Photosynthetic nitrogen use efficiency (PNUE) of *Betula nana* and *Eriophorum vaginatum* across the three fertilization treatments ($N = 4$). Values are means \pm SEM.

is nonmycorrhizal (Chapin et al., 1993; Hobbie and Hobbie, 2006); and (2) their growth forms—*B. nana* is a deciduous shrub requiring rapid and efficient growth during the short growing season (Bret-Harte et al., 2001), and *E. vaginatum* is an evergreen that retains its thick tillers through multiple winters (Fetcher and Shaver, 1983). However, considering these species together, respiration rates corresponded to leaf N and P in a linear manner (Fig. 4), as described previously in plants from different biomes (Reich et al., 1998). Comparison of coupled and uncoupled (maximal) dark respiration rates (Table 3), further emphasize species differences and suggest differing mechanisms under high nutrient fertilization: the small difference between coupled and uncoupled rates in *E. vaginatum* implies little unused respiratory potential, while *B. nana* exhibits the largest difference between coupled and uncoupled rates, perhaps indicating an increased capacity for energy production

and carbon release. The ratios of photosynthesis to respiration also showed significant species effects (Table 1), with *E. vaginatum* demonstrating no variation in net foliar carbon exchange across fertilization levels, while *B. nana* increased from control to the 5N treatment, but decreased from 5N to 10N (Table 2). The decoupled effect of increased respiration and decreased photosynthesis resulted in the lowest net carbon exchange under the highest fertilization, which mirrors measures of net ecosystem exchange from chamber studies (Illeris and Jonasson, 1999; Arens et al., 2008). Therefore, while rates of respiration seem more closely linked to fertilization treatments and foliar N, the lack of a tandem increase in photosynthesis suggests the potential for greater carbon loss from Arctic plants under predicted future environmental conditions.

This study is the first to address the inhibition of respiration by light across a soil nutrient gradient and demonstrates the consistent depression of respiration rates in the light in these species. The general decrease in inhibition with higher soil nutrient availability and leaf N concentration (Fig. 4) supports the idea that inhibition may be relaxed under environmental conditions associated with enhanced photosynthesis to meet the increased cellular energy demand, as observed in *Xanthium strumarium* grown under elevated CO₂ (Wang et al., 2001) and elevated CO₂ and high N availability (Shapiro et al., 2004). Similarly, the lower inhibition and higher respiration rates of *B. nana* may be attributable to its being a deciduous shrub; shrubs allocate a substantial amount of energy expenditure to foliar growth and woody biomass accumulation, which both are enhanced under fertilization during the short Arctic growing season (Bret-Harte et al., 2001, 2002; Shaver et al., 2001). Considering these explanations, it is possible the potential overestimation of leaf and ecosystem respiration when the Kok effect is not measured or incorporated into models may be less pronounced in a future shrubbier, more N-rich tundra system.

Sensitivity of cellular organization to fertilization—In addition to describing the rates of gas exchange, this study also aimed to characterize the underlying cellular organelles that serve as the setting for photosynthesis and respiration and, to our knowledge, represents the only report of these variables in Arctic tundra species and plants under nitrogen and phosphorus treatments. Mitochondria and chloroplasts of mesophyll cells responded to fertilization treatment in terms of density, size, and total cell volume occupied (Table 4), though differed in response patterns between species (Fig. 8). The fine-structure organization of these cells appears to be dynamic and adaptable in both species. Instead of simply increasing the number of mitochondria and chloroplasts as reported in other species grown under elevated CO₂ (Robertson et al., 1995; Griffin et al., 2001; Wang et al., 2004; Gomez-Casanovas et al., 2007), both the size and number of these organelles appear to shift to adapt to the new energy demand. In *B. nana*, this resulted in decoupled increases in organelle total volume that favored chloroplast growth over mitochondrial growth under the highest N and P availability (500% and 27%, respectively), whereas the opposite was the case in *E. vaginatum* (35% and 267%). These contrasting responses of organelle response to fertilization, and therefore assumed energy demand, underscores the differences in short-term adaptations across taxa.

Scaling from the organelles to leaf-level rates of photosynthesis and respiration did not yield obvious functional relationships, though the related increases in total organelle volume

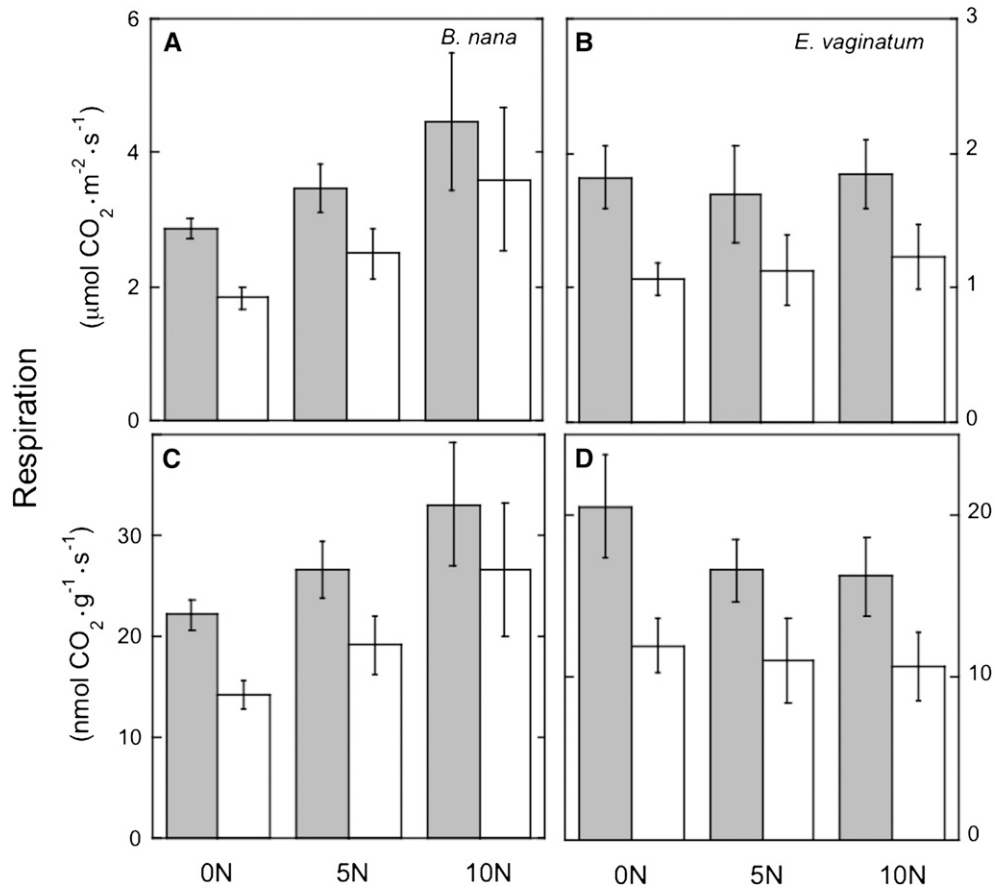


Fig. 6. Area- and mass-based rates of dark respiration (shaded) and respiration in the light (unshaded) from leaves of (A and C) *Betula nana* and (B and D) *Eriophorum vaginatum* grown under fertilization treatments of 0N (control), 5N, and 10N ($N = 4$). Values are means \pm SEM.

per cell corresponded slightly with respiration in both species and with photosynthesis in *E. vaginatum*. However, in *B. nana*, an increase in chloroplast total volume under the highest fertilization did not correlate with the depressed rates of photosynthesis. Comparison of $A : R_L$ and $A : R_D$ with ratios of chloroplasts to mitochondria also showed species differences in organelle-to-process relationships: the maintenance of $A : R$ despite a large drop in the relative total volume of chloroplast to mitochondria in *E. vaginatum* suggests an increased efficiency in chloroplasts under higher N and P availability. This increased efficiency may be due to a restructuring of grana

and stroma thylakoids in response to potential higher photosynthetic activity, as evidenced in leaves under elevated CO_2 (Griffin et al., 2001; Teng et al., 2006). However, in *B. nana* the opposite is evident—an increase in chloroplast to mitochondria ratio was not reflected in the lower $A : R$ ratio under higher fertilization. These discrepancies may be explained by differences in regulation—organelle biogenesis, development, and degradation occur on longer time scales than do photosynthesis and respiration, which can shift according to short-term environmental cues. Also, it is possible that more or larger organelles do not necessarily mean they are all of equal functional efficiency (Jeong et al., 2002). Further, measurement techniques may complicate direct correlations of counts from visualizations of cell fine structure and foliar gas exchange. Organelle measurements made using transmission electron microscopy of ultrathin sections of fixed leaf tissue may limit the potential for direct scaling compared to confocal microscopy, that can localize functionally active mitochondria through staining (Logan and Leaver, 2000; Gomez-Casanovas et al., 2007). This study reports significant relationships in organelle organization under increasing fertilization in two Arctic species that suggest adaptive cellular responses and highlights the potential for future research to further elucidate and quantify the connections between these scales.

Our study incorporates measurements of photosynthesis, respiration, organelle sizes and densities, and leaf nutrients to

TABLE 3. Mean (\pm SE) control and uncoupled (maximal) rates of oxygen consumption ($\text{nmol O}_2 \cdot \text{g DM}^{-1} \cdot \text{s}^{-1}$) in leaf slices of *Betula nana* and *Eriophorum vaginatum*, and the percentage change under uncoupling treatment ($N = 8$).

Treatment	Control	Uncoupled	% Change
<i>B. nana</i>			
0N	5.5 \pm 0.23	6.2 \pm 0.47	+10.83
5N	6.5 \pm 0.63	6.8 \pm 0.41	+4.93
10N	5.4 \pm 0.52	6.9 \pm 0.74	+27.52
<i>E. vaginatum</i>			
0N	6.4 \pm 0.24	7.6 \pm 0.51	+19.56
5N	6.5 \pm 0.51	7.4 \pm 0.41	+14.18
10N	6.8 \pm 0.30	6.8 \pm 0.51	+0.49

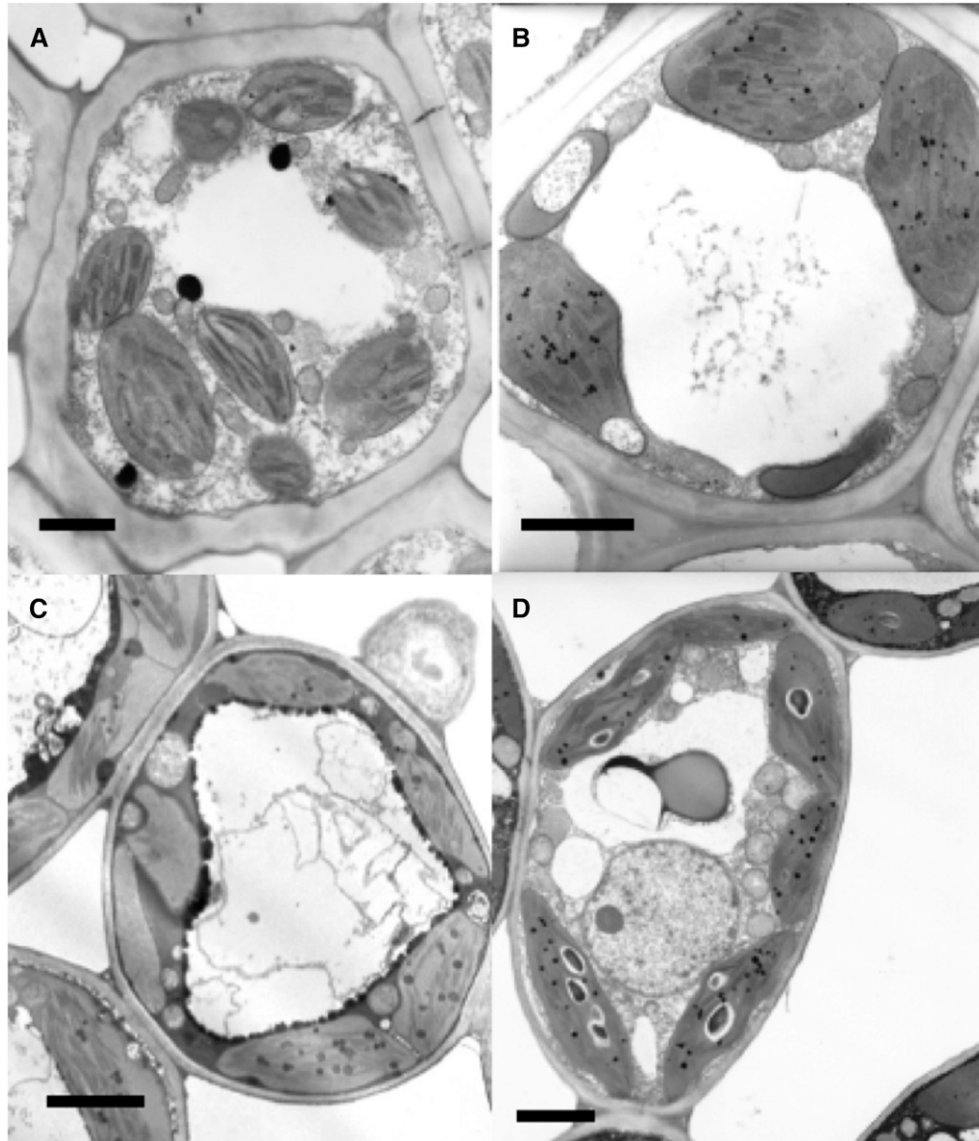


Fig. 7. Chloroplasts and mitochondria in leaf mesophyll cells of *Eriophorum vaginatum* grown under (A) control and (B) 10N fertilization; and *Betula nana* under (C) control and (D) 10N fertilization. Mitochondria are indicated by arrows. Starch granules were present in chloroplasts of *B. nana* under fertilization (D). Scale bars = 2 μ m.

more accurately evaluate leaf-level C cycling in Arctic tundra species under increased N and P availability, as is predicted under warmer temperatures. Results presented highlight the need for leaf-level measurements to describe and predict C cycling

TABLE 4. Results of a two-way ANOVA comparing *Betula nana* and *Eriophorum vaginatum* and fertilization treatment effects on chloroplast (Chloro) and mitochondria (Mito) size ($N = 30$) and density ($N = 45$).

Variable	Species (df = 1)		NP (df = 2)		Species \times NP (df = 2)	
	F-ratio	P	F-ratio	P	F-ratio	P
Chloro density	0.892	0.345	11.160	***	2.684	0.070
Chloro size	7.759	***	8.744	***	23.313	***
Mito density	12.239	***	11.363	***	4.553	*
Mito size	5.039	**	15.851	***	9.707	***

Notes: Significance * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

in a changing environment, especially when considering foliar respiration, which is inhibited by light and can be sensitive to environmental drivers such as N and P fertilization. For accurate estimations of foliar net carbon exchange, the inhibition of respiration of light must be considered, especially in Arctic ecosystems where dark respiration measurements do not reflect reality during the night-less growing season. Neglect of this measurement can lead to inaccurate estimations of C exchange at the leaf, organism, and ecosystem levels. Our study also provides the first known characterizations of mitochondria and chloroplasts in Arctic species, providing further insight on the cellular adaptations to increased nutrient availability. While the decoupled and species-specific variation in foliar C cycling rates under increasing fertilization treatments may complicate predictions, these findings represent another degree of complexity that must be considered in the Arctic tundra under climate change.

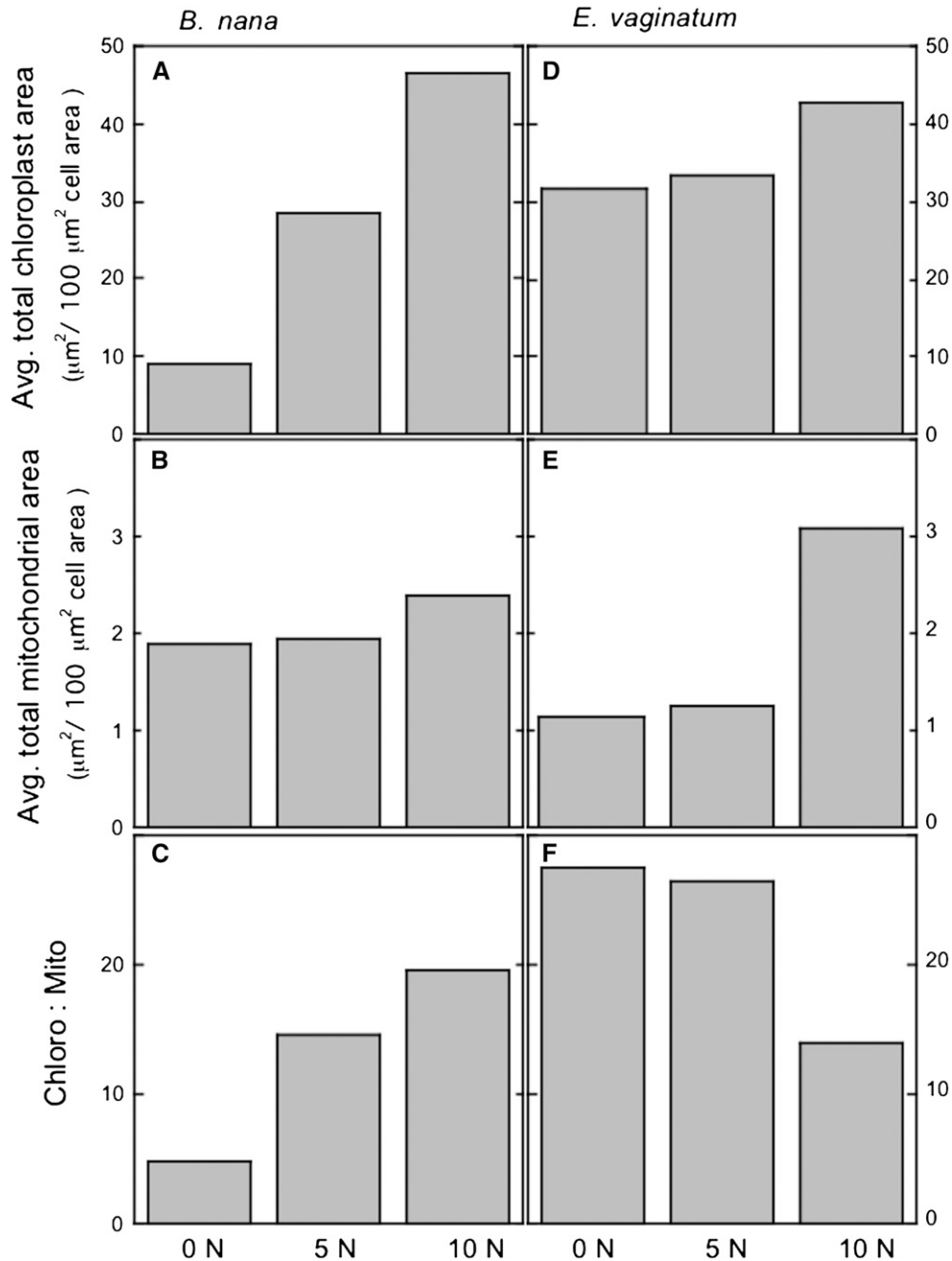


Fig. 8. Average total area of gas exchange organelles per 100 μm^2 mesophyll cell area in (A, B) *Betula nana* and (D, E) *Eriophorum vaginatum*. Values shown are the product of average chloroplast and mitochondria density and average mitochondria and chloroplast size. The ratio of the average total chloroplast area to average total mitochondria area is shown in panels C and F. Error bars are absent for these metrics, as they are calculated based on averages.

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