Host physiological stress enhances hemiparasitism in *Melampyrum lineare* Desr.

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Abstract

Knowledge of the substances transferred in plant hemiparasitic relationships and the factors driving variation in these transfers is limited, especially for hemiparasitic species that are not agricultural pests. We studied the physiology and tissue chemistry of the generalist root hemiparasite *Melampyrum lineare* in a native forest ecosystem where the dominant host species (*Populus grandidentata*) was forced into physiological stress via stem girdling. This experimental treatment decreased belowground carbon (C) allocation by severing the phloem connection with 30% of the canopy leaf area, causing the host root network to mobilize stored C reserves in an attempt to maintain metabolic processes. Decreased starch concentrations in bulk fine roots (-44%) indicated significant solubilization of stored carbohydrates, while decreased sugar concentrations (-18%) suggested that redistribution of stored-C sources was insufficient to meet C demand by belowground sinks. Concurrently, the photosynthetic capacity of *M. lineare* declined significantly in the treatment stand, even as its hypothesized drivers (foliar %N, stomatal conductance, transpiration) were unaffected. However, elevated foliar C concentrations and ^{14}C abundances suggested that *M. lineare* in the treatment stand had accumulated solubilized, fixed C that was being redistributed within host root tissues, causing downregulation of photosynthetic capacity in this annual, facultative hemiparasitic species. In treatment stand *M. lineare*, a positive correlation between foliar %C and $\Delta^{14}C$ indicated an increasing contribution of C from host-derived (storage) sources as foliar C concentrations increased, and a similar relationship between foliar %C and $\delta^{15}N$ suggests that as *M. lineare* received increasing amounts of stored host root carbohydrates, rates of nitrate-derived N losses from host rhizospheres increased. We conclude that

47 photosynthetic regulation by *M. lineare* is sensitive to C transfer from host roots, a
48 process that primarily occurs during conditions of physiological stress that cause
49 remobilization of stored carbohydrates. As a C sink that competes with other root
50 metabolic demands, hemiparasitic C drain may impact the ability of stressed host roots to
51 provide sufficient C for N uptake, causing changes in N cycling and retention within host
52 rhizospheres.
53
54 **Keywords:** root hemiparasitism, carbon, nitrogen, photosynthesis, carbohydrate, ^{14}C,
55 ^{13}C, ^{15}N
56
57 **Introduction**
58 Root hemiparasitic plant species are a small component of the global flora but are widely
59 distributed across terrestrial ecosystems. Available evidence suggests hemiparasitic
60 plants have impacts on biogeochemical cycling, plant demography and diversity that are
61 disproportionately large relative to their abundance and biomass within ecosystems
62 (Quested et al., 2003; Stewart and Press, 1990), however most information concerning
63 their role in ecosystems has been developed from studies on crop pests and Eurasian taxa
64 including *Striga* spp. (Lour.) and *Rhinanthus minor* (L.). Such studies have identified
65 compounds that hemiparasites derive from their hosts and quantified rates of
66 hemiparasites' physiological processes as a function of host identity (Pennings and
67 Callaway, 2002). There has been considerably less research on host-hemiparasite
68 relations among North American taxa, and few studies have used an experimental
69 approach to investigate how root hemiparasites and their hosts operate as coupled

systems wherein the hemiparasite registers biogeochemical changes at scales larger than the individual organism (*e.g.*, Hattenschwiler and Korner, 1997). Experimental work that elucidates the biogeochemical relationships between hemiparasitic plants and their hosts in the wild can therefore add much context to studies that emphasize physiological processes and agriculturally important taxa.

Root hemiparasites generally obtain xylem water and mineral nutrients through their haustoria, which penetrate into host root steles (Seel and Press, 1993). Root hemiparasites are fully capable of photosynthesis, and rates typically increase with host attachment, which can provide fixed C in addition to water and mineral nutrients (Seel and Press, 1994; Press et al., 1987). Research with several Eurasian taxa has demonstrated that host identity affects root hemiparasite physiology through interspecific variation in host tissue chemistry and water use (Seel and Press, 1993; 1994; Seel et al., 1993), posing the question of whether changes in host biogeochemistry occurring at stand-to-ecosystem levels can also modulate hemiparasite physiology.

Melampyrum lineare Desr. (Orobanchaceae) is a facultative, root hemiparasitic annual widely distributed across North America, where it forms haustorial connections with a variety of different host species (Bennet and Matthews, 2006; Cantlon et al., 1963). *M. lineare* is myrmecochorous, leading to nonrandom seed dispersal along travel routes favored by ants, who collect and discard the seeds after extracting their nourishing elaiasomes (Gibson, 1993). Following germination, preparasitic *M. lineare* seedlings infect nearby host roots with their haustoria. The full suite of compounds obtained

through haustoria is not known for *M. lineare*, but includes water and P (Cantlon et al., 1963). Based on work with Eurasian *Melampyrum* spp., it is probable that *M. lineare* also acquires N, and possibly carbohydrates through its hemiparasitic habit (Gauslaa, 1990; Hattenschwiler and Korner, 1997; Lechowski, 1996). In the Great Lakes region of North America, *M. lineare* is an abundant species in forests dominated by *Pinus* spp*., Quercus* spp*.,* and *Populus* spp. (Cantlon et al., 1963). In many of these forests, senescence of dominant, short-lived *Populus* spp. is causing succession towards longer-lived trees, suggesting the possibility that biogeochemical shifts accompanying *Populus* mortality (Nave et al., 2011) may impact the physiology of the intimately associated root hemiparasite *M. lineare*. We investigated the impact of stand-level changes in host biogeochemistry on *M. lineare* physiology using an ecosystem-scale experimental manipulation that accelerated the senescence of *Populus grandidentata* Michx., the dominant canopy species, by disrupting its ability to transport carbohydrates belowground. Following on other changes observed with this experiment, we hypothesized that the herbaceous annual *M. lineare* would respond to a period of elevated soil N availability with higher foliar [N] and increased photosynthetic capacity. Upon rejecting this hypothesis, we generated an alternate hypothesis - that stored root C was flowing from host *P. grandidentata* into *M. lineare* and causing photosynthetic downregulation- and tested its predictions with environmentally replicated measurements of ^{14}C abundance.

Methods

Study Site

116 We conducted this study at the University of Michigan Biological Station (UMBS) in
117 northern Lower Michigan, USA (45°35.5'N 84°43'W), which has a temperate continental
118 climate with a strong Great Lakes influence (MAT=5.5° C, MAP=817 mm with annual
119 snowfall of 294 cm and a snowpack typically from December until early April). The
120 study area is on an outwash plain where soils are well-drained Haplorthods with forest
121 floors consisting of Oe horizons 1-3 cm thick overlying bioturbated AO horizons of 1-3
122 cm held together by dense fine roots. The remainder of the soil profile within the
123 predominant rooting zone includes an E horizon of 10-15 cm and a Bs horizon of sand
124 with occasional gravel and cobble. These coarse-textured soils (>95% sand) have
125 relatively low pH, very low organic matter and N availability (Nave et al., 2011). In the
126 study area, stem density of trees > 8 cm dbh is 700-800 ha^{-1} and leaf area index (LAI)
127 averages 3.5 m^2 m^{-2}. *P. grandidentata* is the canopy dominant of the secondary
128 successional forest within the study area, which averages 85-90 yr in age. Other canopy
129 species include *Acer rubrum* L., *Quercus rubra* L., *Betula papyrifera* Marsh., *Pinus*
130 *strobus* L., *Populus tremuloides* Michx., *Acer saccharum* Marsh., and *Fagus grandifolia*
131 Ehrh.. The understory is dominated by *A. rubrum*, *Q. rubra*, *P. strobus*, and *Amelanchier*
132 spp., while *Pteridium aquilinum* L. and *Vaccinium angustifolium* Aiton are the most
133 abundant ground flora. Forest composition and disturbance history at the study site are
134 representative of upland forests throughout the northern Great Lakes region, where
135 *Populus*-dominated northern hardwoods replaced old-growth *Pinus-Tsuga* forests
136 following clearcutting and wildfires in the late 19th and early 20th centuries (Gough et al.,
137 2007).
138

We performed sampling activities for this study in two large (1.1 ha), intensively measured permanent plots located approximately 2 km apart in forest stands of average soil fertility, aboveground biomass, and species composition relative to the surrounding landscape. One of the plots is situated within a 30 ha experimental area in which all *Populus* spp. and *B. papyrifera* individuals (30% of LAI) were stem girdled in April-May of 2008 as part of the Forest Accelerated Succession ExperimenT (FASET). FASET is tracking changes in ecosystem function as this widespread forest type undergoes transition from early to later successional plant communities; broader biogeochemical changes ongoing at the time of this study are described in Nave et al. (2011).

Selection of M. lineare *plants for sampling*

We randomly established 24 sampling locations within each plot, and at each location we found and flagged the 3 nearest *M. lineare* plants (=72 total plants per plot). Plant selection was random with regard to size, except in infrequent cases when leaves were less than 2 cm in length, making them too small to reach across the length of a standard 2 x 3 cm leaf gas exchange cuvette. In such cases, we located the next closest plant with sufficiently large leaves.

Leaf gas exchange

We conducted measurements of light-saturated photosynthetic capacity, stomatal conductance, and transpiration on a random subset (18) of the 72 *M. lineare* plants in each plot using a LI-COR 6400 Portable Photosynthesis System (LI-COR, Lincoln, NE, USA). We made these measurements on 7 July 2009, visiting 9 of the plants in each plot

during one of two time periods (0800 to 1200, 1400 to 1700). We distributed measurements between these two time periods to control for potential time-of-day effects through a randomized, balanced design blocked by time (AM vs. PM). On each *M. lineare* individual, we measured gas exchange parameters of the youngest fully expanded leaf long enough to fit perpendicularly across the 2 x 3 cm cuvette. After the 30-90 second interval required to obtain stable readings, we removed the leaf from the plant, calculated its area from Vernier caliper measurements, and scaled all gas exchange measurements to the full area of the cuvette. Cuvette conditions were held at 1500 μmol m^{-2} s^{-1} photosynthetic photon flux density, 385 µmol mol^{-1} [CO_2], 25 °C temperature, and > 65% humidity.

Tissue chemistry

On 9 July 2009, we harvested the aboveground portions of all 72 *M. lineare* plants from each plot. We made these collections during a brief time period (1300 to 1600) to minimize the possibility of diurnal variation in tissue chemistry. At each of the 24 sampling points from which we collected these plants, we pooled the aboveground portions of all 3 individuals into one sample and oven-dried all such samples at 60°C overnight. Next, we removed the leaves from the stems, ground the leaves in a ball mill and weighed them into tin capsules for %C, %N, $\delta^{13}C$, and $\delta^{15}N$ analysis on a Costech Analytical CHN analyzer (Costech Analytical, Valencia, CA USA) coupled to a Finnegan Delta Plus XL isotope ratio mass spectrometer (Thermo Scientific, West Palm Beach, FL USA) in the UMBS analytical lab. Instrument error as checked by internal standards was 0.1‰ for $\delta^{15}N$ and $\delta^{13}C$, 0.1% for %N, and 0.6% for %C (standard

185 deviations). We obtained ^{14}C abundance measurements for each *M. lineare* sample via
186 graphitization according to standard methods (Vogel et al., 1987) and measurement at the
187 Center for Accelerator Mass Spectrometry, Lawrence Livermore National Lab (Davis et
188 al., 1990). $\Delta^{14}C$ values include a background subtraction determined from ^{14}C-free coal
189 and a $\delta^{13}C$ correction to account for isotopic fractionation (Stuiver and Polach, 1977).
190 Analytical error on average was 3.2‰ $\Delta^{14}C$.
191
192 We collected bulk forest floor fine roots to assess the effects of stem girdling on the fine
193 root network that hosts the generalist hemiparasite *M. lineare*. We used a 7 cm diameter
194 corer to obtain forest floor monoliths from beneath harvested *M. lineare* plants
195 immediately following *M. lineare* aboveground biomass collection, and pooled the
196 individual monoliths into composite samples (similar to the aboveground biomass
197 samples). We removed mineral contamination from the forest floor samples by rinsing
198 over a 1mm mesh screen, then floated each forest floor sample in water and removed all
199 roots 0.5 – 2.0mm diameter, which were lyophilized and ground with a ball mill.
200 Powdered fine root samples were stored at -80° until beginning nonstructural
201 carbohydrate analysis following the methods of Curtis (2000), which involved extracting
202 soluble sugars with ethanol, digesting residual starch, and assaying the concentrations of
203 both carbohydrate fractions on a Spectronic Genesys 2 spectrophotometer (Spectronic
204 Analytical Instruments, Leeds, UK). Both carbohydrate pools were converted to a % of
205 fine root dry mass basis for data analysis.
206
207 *Data analysis*

We tested for significant treatment effects on *M. lineare* foliar C and N concentrations, isotopic abundances, and bulk fine root carbohydrate concentrations using t-tests, after confirming validity of crucial parametric assumptions (we considered moderate deviations from normality acceptable). We tested for overall treatment effects on *M. lineare* leaf gas exchange parameters using ANOVA (blocked by time) after verifying parametric assumptions. We conducted these categorical analyses using SPSS (IBM Corp., Armonk, NY USA), and assessed continuous relationships between *M. lineare* foliar C concentrations and isotopic values using simple linear regression with SigmaPlot (SYSTAT Software, San Jose, CA USA). For all tests in this field ecological study, we chose at the time of initial study design to accept results as significant if $P < 0.10$.

Results

M. lineare photosynthetic capacity was significantly lower in the treatment stand than in the control stand (Figure 1), although none of the typical drivers of photosynthetic capacity responded to experimental treatment. Specifically, there was no significant difference in *M. lineare* foliar N concentration (3.0 vs. 3.1% in control and treatment stands, respectively), stomatal conductance (0.15 vs. 0.13 mmol s^{-1}), or transpiration rate (1.9 vs. 1.6 mol H_2O m^{-2} s^{-1}). However, *M. lineare* foliar C concentrations, ^{15}N and ^{14}C abundances were significantly higher in the treatment stand (Table 1), where foliage with higher C concentrations had increasing abundances of ^{15}N and ^{14}C (Figure 2). Bulk forest floor fine roots had significantly lower soluble sugar and starch concentrations in the treatment stand than the control stand (Table 2).

Discussion

The decreased photosynthetic capacity of treatment stand *M. lineare* was contrary to our initial hypothesis and not related to any of the usual drivers of leaf-level photosynthesis, *i.e*., hydraulic or N-limitation (Reich et al., 1997). However, in the context of its significantly elevated foliar C concentration, the decreased photosynthetic capacity of treatment stand *M. lineare* is consistent with the idea of a sink-induced photosynthetic downregulation, driven by accumulation of photosynthetic end products such as soluble carbohydrates (Goldschmidt and Huber, 1992; Paul and Foyer, 2001).

In light of the accelerated stored-C catabolism occurring in the roots of girdled *P. grandidentata* during this experiment, our results suggest that the C accumulated in treatment stand *M. lineare* foliage was derived from a steady transpiration stream containing parasitized carbohydrates from the roots of these senescing, dominant host trees. Stem girdling physically prevented allocation of new photosynthate belowground by *P. grandidentata* trees, causing their extensive clonal root network to mobilize stored carbohydrates in an attempt to maintain metabolic processes and turnover. In *Populus*, stored-C mobilization typically involves breakdown of starch reserves to maintain a pool of soluble sugars, which may be transported throughout the root system to sustain metabolic requirements (Ländhausser and Lieffers, 2012; Regier et al., 2010). The large decrease in root starch concentrations in the treatment stand indicate that this mobilization process was ongoing and had depleted almost half of stored reserves at the time of sampling, while the relatively smaller (but still significant) decrease in soluble sugars indicates that these carbohydrates were consumed by sinks including root

254 respiration, turnover, and *M. lineare* haustoria faster than they could be mobilized from
255 starch reserves.
256
257 The increasing abundance of ^{14}C with *M. lineare* foliar C concentration in the treatment
258 stand reaffirms the idea that solubilized, stored *P. grandidentata* carbohydrates were a
259 significant source of C to *M. lineare*. This stored C that was transferred to *M. lineare*
260 possesses ^{14}C values that reflect the steadily declining 'bomb curve' of ^{14}C abundance in
261 the atmosphere (Hua and Barbetti, 2004). Due to the steady decline of the atmospheric
262 radiocarbon signature since the mid-1960s, photosynthates fixed in previous years
263 contain more radiocarbon (*i.e.* higher $\Delta^{14}C$ value) than more recently fixed
264 photosynthates. In response to this influx of previously fixed C from stored host sources,
265 *M. lineare* downregulated its photosynthetic capacity, a physiological adjustment that has
266 also been documented in the agricultural host-hemiparasite system of *Triticum aestivum*
267 L. - *Thesium humile* Vahl. (Santalaceae; Fer et al., 1993). Together, these observations
268 form a pattern consistent in native and agricultural ecosystems and across hemiparasitic
269 families- namely, that photosynthesis and the internal C sink strength of hemiparasitic
270 plants are responsive to C supply by host roots.
271
272 The positive relationship between *M. lineare* foliar C concentration and $\delta^{15}N$ in the stem-
273 girdled stand suggests that as hemiparasitic uptake of carbohydrates from senescing host
274 roots increased, so did loss rates of isotopically depleted, nitrate-derived N from host root
275 rhizospheres. We have interpreted ^{15}N enrichments of tree foliage (Nave et al., 2011) and
276 ectomycorrhizal sporocarps (Nave and Nadelhoffer, unpublished data) during this

277 experiment as indicators of nitrate-derived N losses that occurred due to leaching and
278 gaseous efflux (*sensu* Högberg, 1990). N losses via these pathways, which did not occur
279 before stem girdling, occurred because the solubilization of stored root carbohydrates and
280 their redistribution within the xylem stream could not provide *P. grandidentata* with
281 enough C skeletons for root N assimilation. With this result, it appears that *M. lineare*
282 hemiparasitism could have been a sufficiently significant sink for a limited stored-C
283 supply to contribute to the host species' inability to take up N.
284
285 It is significant that the ^{14}C and ^{15}N isotopic patterns in *M. lineare* from the
286 experimentally treated stand (increasing abundances with foliar %C) do not hold for *M.*
287 *lineare* from the control stand. At the time of this experiment, normal metabolic
288 functioning of host (*P. grandidentata*) fine roots in the control stand was maintained by
289 current photosynthate (Nave et al., 2011). In the absence of stem girdling, these host roots
290 were themselves the C sink in a phloem-coupled relationship with the photosynthetic
291 canopy, so there was no metabolic demand for redistribution of stored carbohydrates
292 within the transpiration-driven xylem stream. We therefore suggest that while
293 hemiparasitism by *M. lineare* is responsive to host C supply it is nonetheless inherently
294 facultative; the hemiparasite does not actively 'steal' C from its host but rather acts as a C
295 sink during times of host stress that lead to stored-C catabolism and redistribution within
296 the root system.
297
298 *Conclusion*

We used an ecosystem-scale experimental manipulation to create an advantageous set of conditions for studying the biogeochemical relationship between the hemiparasite *M. lineare* and its hosts. By interrupting belowground C allocation by the dominant canopy species, stem girdling induced the mobilization of stored carbohydrates within the host root network, and resulted in an increased supply of soluble C from these host roots that led the facultative hemiparasite *M. lineare* to downregulate its photosynthetic capacity. Correlations between *M. lineare* foliar C concentrations, ^{14}C and ^{15}N abundances in the treatment stand (but not the control stand) confirmed our proposed mechanism for photosynthetic downregulation, and indicate that this process is only likely to occur when hosts are stressed, physiological function is impaired, and reliance on stored root carbohydrates increased.

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Literature Cited

322 Bennet, J.R., and S. Matthews. 2006. Phylogeny of the parasitic plant family
323 Orobanchaceae Inferred from Phytochrome A. American Journal of Botany 93(7):
324 1039-1051.
325 Cantlon, J. E., E.J.C Curtis, and W.M. Malcolm. 1963. Studies of *Melampyrum lineare*.
326 Ecology 44(3): 466-474.
327 Curtis, P.S., C.S. Vogel, X. Wang, K.S. Pregitzer, D.R. Zak, J. Lussenhop, M. Kubiske,
328 and J. A. Teeri. 2000. Gas exchange, leaf nitrogen, and growth efficiency of
329 Populus tremuloides in a CO_2 enriched atmosphere. Ecological Society of
330 America 10(1): 3-17.
331 Davis, J.C., I.D. Proctor, J.R. Southon, M.W. Caffee, D.W. Heikkinen, M.L. Roberts,
332 T.L. Moore, K.W. Turteltaub, D.E. Nelson, D.H. Loyd, and J.S. Vogel. 1990.
333 LLNL/US AMS facility and research program. Nuclear Instruments and Methods
334 in Physics Research, Sect. B 52: 269-272.
335 Fer, A., P. Simier, M.C. Arnaud, L. Rey, and S. Renaudin. 1993. Carbon acquisition and
336 metabolism in a root hemiparasitic angiosperm, *Thesium humile* (Santalaceae)
337 growing on wheat (*Triticum vulgare*). Australian Journal of Plant Physiology 20,
338 15-24.
339 Gauslaa, Y. 1990. Water Relations and Mineral Nutrients in *Melampyrum pratense*
340 (Scrophulariaceae) in oligotrophic and mesotrophic boreal forests. Acta
341 Oecologica-International Journal of Ecology 11, 525-537.
342 Gibson, W. 1993. Selective advantages to hemiparasitic annuals, genus *Melampyrum*, of
343 a seed-dispersal mutualism involving ants. 1. Favorable nest sites. Oikos 67, 334-
344 344.

345 Goldschmidt, E.E., and S.C. Huber. 1992. Regulation of photosynthesis by end-product
346 accumulation in leaves of plants storing starch, sucrose, and hexose sugars. Plant
347 Physiology 99, 1443-1448.
348 Gough, C. M., C. S. Vogel, K. H. Harrold, K. George, and P. S. Curtis 2007. The legacy
349 of harvest and fire on ecosystem carbon storage in a north temperate forest.
350 Global Change Biology 13, 1935–1949,
351 Hattenschwiler, S and C. Korner. 1997. Growth of autotrophic and root-hemiparasitic
352 understory plants under elevated CO_2 and increased N deposition. Acta
353 Oecologica-International Journal of Ecology 18, 327-333.
354 Högberg, P. 1990. Forests losing large quantities of nitrogen have elevated N-15:N-14
355 ratios. Oecologia 84, 229-231.
356 Hua, Q., and M. Barbetti. 2004. Review of Tropospheric Bomb 14C Data for Carbon
357 Cycle Modeling and Age Calibration Purposes. Radiocarbon 46(3): 1273-1298.
358 Ländhausser, S.M., and V.J. Lieffers. 2012. Defoliation increases risk of carbon
359 starvation in root systems of mature aspen. Trees 26: 653-661.
360 Lechowski, Z. 1996. Gas exchange in leaves of the root hemiparasite *Melampyrum*
361 *arvense* L. before and after attachment to the host plant. Biologia Plantarum 38,
362 85-93.
363 Nave, L.E., C.M. Gough, K.D. Maurer, G. Bohrer, B.S. Hardiman, J. Le Moine, A.B.
364 Muñoz, K.J. Nadelhoffer, J.P. Sparks, B.D. Strahm, C.S. Vogel, and P.S. Curtis.
365 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a
366 northern temperate forest. Journal of Geophysical Research- Biogeosciences 116,
367 G04016.

368 Paul, M.J., and C.H. Foyer. 2001. Sink regulation of photosynthesis. Journal of
369 Experimental Botany 52, 1383-1400.
370 Pennings, S.C., and R.M. Callaway. 2002. Parasitic plants: parallels and contrasts with
371 herbivores. Oecologia 131, 479-489.
372 Press, M.C., N. Shah, J.M. Tuohy, and G.R.Stewart. 1987. Carbon isotope ratios
373 demonstrate carbon flux from C-4 host to C-3 parasite. Plant Physiology 85,
374 1143-1145.
375 Quested, H.M., J.H.C. Cornelissen, M.C. Press, T.V. Callaghan, R. Aerts, F. Trosien, P.
376 Riemann, D. Gwynn-Jones, A. Kondratchuk, and S.E. Jonasson SE. 2003.
377 Decomposition of sub-arctic plants with differing nitrogen economies: A
378 functional role for hemiparasites. Ecology 84, 3209-3221.
379 Reich, P.B., M.B. Walters, and D.S. Ellsworth. 1997. From tropics to tundra: Global
380 convergence in plant functioning. Proceedings of the National Academy of
381 Sciences of the United States of America 94, 13730-13734.
382 Regier, N., S. Streb, S.C. Zeeman, and B. Frey. 2012. Seasonal changes in starch and
383 sugar content of poplar (*Populus deltoides* x nigra cv. Dorskamp) and the impact
384 of stem girdling on carbohydrate allocation to roots. Tree Physiology 30:979-987.
385 Seel, W.E., R.E. Cooper, and M.C. Press. 1993. Growth, gas exchange and water use
386 efficiency of the facultative hemiparasite *Rhinanthus minor* associated with hosts
387 differing in foliar nitrogen concentration. Physiologia Plantarum 89, 64-70.
388 Seel, W.E., and M.C. Press. 1993. Influence of the host on 3 subarctic annual facultative
389 root hemiparasites. 1. Growth, mineral accumulation and aboveground dry matter
390 partitioning. New Phytologist 125, 131-138.

Seel, W.E., and M.C. Press. 1994. Influence of the host on 3 subarctic annual facultative root hemiparasites. 2. Gas exchange characteristics and resource use efficiency. New Phytologist 127, 37-44.

Stewart, G.R., and M.C. Press. 1990. The physiology and biochemistry of parasitic angiosperms. Annual Review of Plant Physiology and Plant Molecular Biology 41, 127-151.

Stuiver, M., and H.A. Polach, 1977. Discussion: reporting of ^{14}C data. Radiocarbon 19: 355-363.

Vogel, J.S., J.R. Southon, and D.E. Nelson. 1987. Catalyst and binder effects in the use of filamentous graphite for AMS. Nuclear Instruments and Methods in Physics Research Sect. B 29: 50-56.

Table 1. Foliar C and N concentrations, ^{15}N and ^{14}C abundances of *M. lineare* from control and treatment stands. Data are means ±SE. For each response parameter, asterisks denote the level of statistical significance for the difference between means (*P <.10; **P<.05; ***P <.01).

Group	%N	%C**	$\delta^{15}N$***	$\Delta^{14}C$**
Control	3.0 ±0.08	**38.9 ±0.3**	**-6.1 ±0.18**	**41.6 ±1.1**
Treatment	3.1 ±0.08	**39.8 ±0.3**	**-4.1 ±0.17**	**44.9 ±1.1**

Table 2. Soluble sugar and starch concentrations of bulk forest floor fine roots from control and treatment stands. Data are means ±SE. For each response parameter, asterisks denote the level of statistical significance for the difference between means (see Table 1 for definitions).

Group	Soluble sugars**	Starch**
Control	**1.8 ±0.1**	**7.3 ±1.3**
Treatment	**1.4 ±0.1**	**4.1 ±0.7**

Figure legends

Figure 1. Light-saturated photosynthesis of *M. lineare* in non-girdled (control) and stem-girdled (treatment) aspen stands. Bars are means ±SE; *P* value indicates the level of statistical significance for the difference between means.

Figure 2. Relationships between the C concentration, ^{14}C (panel a; top) and ^{15}N (panel b; bottom) abundances of *M. lineare* foliage. In each panel, the filled points, best-fit lines, and regression *P* values correspond to *M. lineare* from the treatment stand. Open points with error bars show the mean ±SE (n=24) tissue chemistry of control stand *M. lineare* for reference; these plants showed no significant isotopic relationships with foliar C concentration.

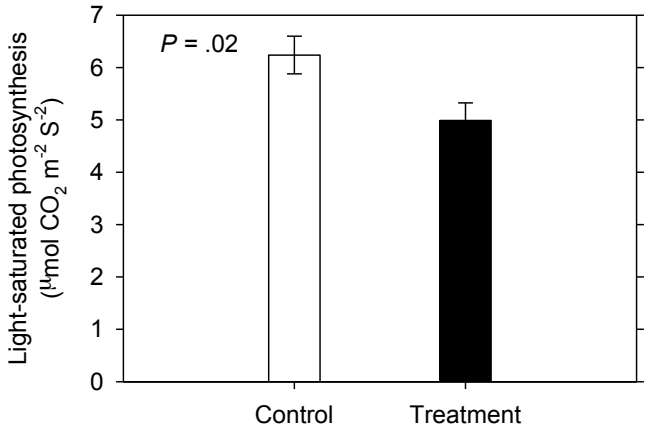
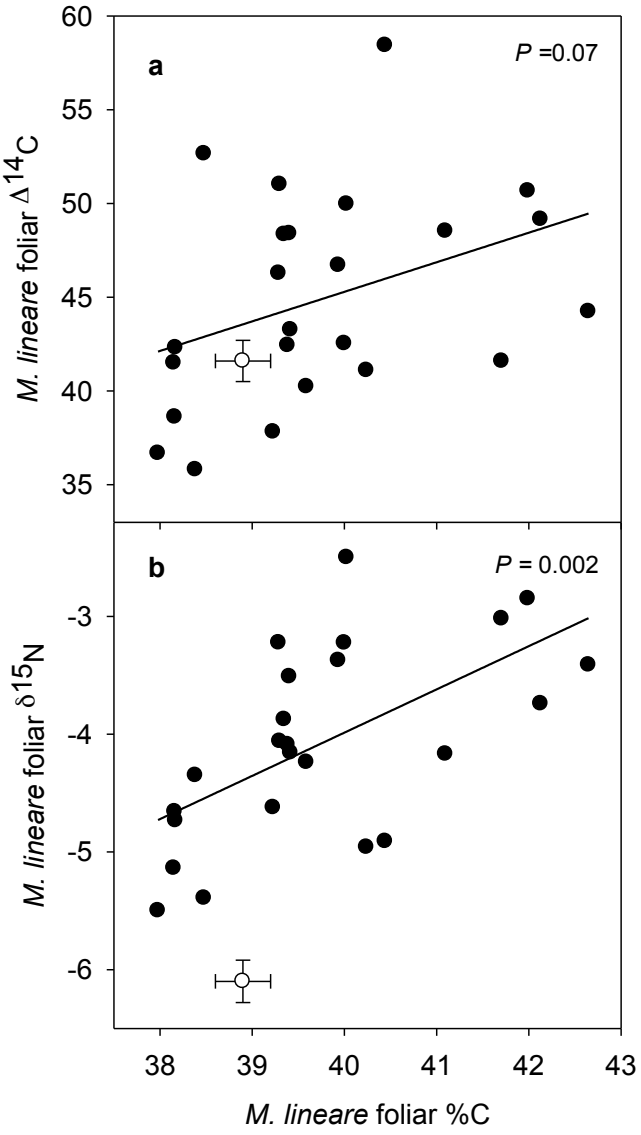

P = .02
Light-saturated photosynthesis
(μmol CO2 m-2 S-2)
0
1
2
3
4
5
6
7
Control
Treatment

Figure 1.



490

491 Figure 2.

492

493