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Phosphorus addition increased carbon partitioning to autotrophic respiration but not to biomass production in an experiment with Zea mays

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3 experiment with *Zea mays*

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6 P effects on plant C partitioning
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ABSTRACT

Plant carbon (C) partitioning - the relative use of photosynthates for biomass production, respiration, and other plant functions - is a key but poorly understood ecosystem process. In an experiment with *Zea mays*, with or without arbuscular mycorrhizal fungi (AMF), we investigated the effect of phosphorus (P) fertilization and AMF on plant C partitioning. Based on earlier studies, we expected C partitioning to biomass production (i.e., biomass production efficiency; BPE) to increase with increasing P addition due to reduced C partitioning to AMF. However, although plant growth was clearly stimulated by P addition, BPE did not increase. Instead, C partitioning to autotrophic respiration increased. These results contrasted with our expectations and with a previous experiment in the same set-up where P addition increased BPE while no effect on autotrophic respiration was found. The comparison of both experiments suggests a key role for AMF in explaining these contrasts. Whereas in the previous experiment substantial C partitioning to AMF reduced BPE under low P, in the current experiment, C partitioning to AMF was too low to directly influence BPE. Our results illustrate the complex influence of nutrient availability and mycorrhizal symbiosis on plant C partitioning.

KEY WORDS

arbuscular mycorrhizal fungi, autotrophic respiration, biomass production efficiency, carbon allocation, carbon balance, carbon partitioning, carbon use efficiency, phosphorus

INTRODUCTION

Plants take up carbon (C) from the atmosphere through photosynthesis (gross primary production, GPP). They allocate this C either to various organs and convert it into plant biomass production, export it to the rhizosphere (e.g. through symbiosis with mycorrhizal fungi) or use it to meet their energy requirements (Churchland *et al.*, 2013; Vicca *et al.*, 2012). This partitioning of assimilated C among functions determines the C residence time in the ecosystem and ultimately reflects the ecosystem C sink strength. It is a key ecosystem process that affects terrestrial biogeochemistry, through influences on litter quality and decomposition rates, C sequestration, and plant–atmosphere gas exchange (Cropper & Gholz, 1994; Friedlingstein *et al.*, 1998; Bird & Torn, 2006).

Plant C partitioning can vary strongly (Litton *et al.*, 2007; Verlinden *et al.*, 2018). While in some ecosystems C partitioning to plant biomass is only about 30%, others use up to 70% of the photosynthates for biomass production (DeLucia *et al.*, 2007; Litton *et al.*, 2007; Vicca *et al.*, 2012; Raich *et al.*, 2014; Campioli *et al.*, 2015). It is not clear yet why this variation occurs, but evidence is growing that nutrient availability plays a key role (Vicca *et al.*, 2012; Buendía *et al.*, 2014; Collalti & Prentice, 2019).

Phosphorus (P) and nitrogen (N) are globally the most limiting nutrients for plant growth (Elser *et al.*, 2007; LeBauer & Treseder, 2008), and variation in their availability can strongly impact plant C partitioning. For example, annual plant growth is typically more responsive to variation in nutrient availability than photosynthesis resulting in enhanced ratio of total plant biomass production to photosynthesis (i.e. biomass production efficiency; BPE) with increasing nutrient availability (e.g. Vicca *et al.*, 2012; Verlinden *et al.*, 2018). And while partitioning to aboveground growth usually increases with increasing P and N availability, belowground C partitioning generally decreases (Ericsson, 1995; Lilleskov *et al.*, 2002; Treseder, 2004; Vicca *et al.*, 2012), as plants then need to invest less to acquire P (Ekblad *et al.*, 1995; Johnson, 2010).

While both N and P are important regulators of C partitioning, their influence remains poorly quantified (Treseder, 2004; Gill & Finzi, 2016; Verlinden *et al.*, 2018).

Plants respire a large fraction of their GPP, with autotrophic respiration relative to GPP ($R_{\text{aut}}:GPP$) typically around 0.5 (Collalti & Prentice, 2019). From a plant physiology perspective it is often argued that autotrophic respiration is a relatively constant fraction of GPP due to interdependencies of respiration and photosynthesis (Dewar *et al.*, 1998; Hoefnagel *et al.*, 1998; van Oijen *et al.*, 2010). Several studies have confirmed a relatively constant respiration-to-photosynthesis ratio across species and ecosystems (Reich *et al.*, 1998; Loveys *et al.*, 2003; Turnbull *et al.*, 2005), but deviations from this pattern have also been observed, especially when circumstances were more extreme (e.g. at high temperatures; Atkin *et al.*, 2007; Campbell *et al.*, 2007; Drake *et al.*, 2016). Although the influence of nutrient availability on C partitioning to R_{aut} is still poorly understood, the limited evidence available suggests no effect of nutrient availability on $R_{\text{aut}}:GPP$ (Vicca *et al.*, 2012 and references therein; Verlinden *et al.*, 2018). However, generalizations about influences of nutrients on $R_{\text{aut}}:GPP$ are often impeded primarily due to limited availability of accurate and mutually independent quantifications of both biomass production (BP) and R_{aut} (Collalti & Prentice, 2019).

The majority of vascular plants live in symbiosis with AMF, where plants provide C by transferring carbohydrates via roots, while AMF supply their host with nutrients, especially P (Smith & Read, 2008; Brundrett, 2009; Johnson, 2010). One of the more widely tested hypotheses within the field of mycorrhizal ecology is that mycorrhizal fungi grow better and are more abundant when nutrient availability is low (Mosse & Phillips, 1971); when N or P availability rises, plants invest less C in nutrient acquiring mechanisms, which generally reduces mycorrhizal abundance (Treseder, 2004). This response is not universal, however, and N or P addition can even increase AMF growth under (strongly) nutrient-limited conditions (Treseder & Allen, 2002; Raven *et al.*, 2018). Given their central role in plant nutrient

acquisition, also plant C partitioning is expected to be influenced by AMF. For example, a lower C cost for mycorrhizal symbiosis with increasing nutrient availability has been suggested as the underlying mechanism explaining the typical increase of BPE with increasing nutrient availability (Jakobsen & Rosendahl, 1990; Vicca *et al.*, 2012; Verlinden *et al.*, 2018). However, this remains to be verified empirically.

We set up a fertilization experiment with a soil originally limiting in P but not in N to investigate the effect of different levels of P fertilization on plant C partitioning, including BPE and $R_{\text{aut}}:GPP$. We also compared mesocosms with and without AMF. The main hypothesis was that BPE increases with increasing P fertilization due to reduced investment in AMF symbiosis, while $R_{\text{aut}}:GPP$ was expected to be relatively constant among treatments. A similar experiment was conducted one year earlier, using the same set-up, but with N, P and N + P fertilization instead of a P gradient (Verlinden *et al.*, 2018). This experiment showed that the growth of maize in these soils was strongly limited by P availability, while N was ample as N addition did not affect plant growth (Verlinden *et al.*, 2018). In the current study, we therefore opted for a P gradient instead of an NxP experiment; gradient studies are also especially useful for establishing response functions that help to better constrain vegetation models (Vicca *et al.*, 2018). We also compare both experiments to gain better insight into plant C partitioning and the role of AMF.

MATERIALS AND METHODS

Experimental set-up

The set-up consisted of 30 insulated mesocosms (1 m × 1.2 m × 0.6 m high). The set-up and soil mixture were the same as in Verlinden *et al.* (2018). For more information, see Appendix S1. The soil in each mesocosm from the previous experiment (Verlinden *et al.*, 2018) could be used to start the current experiment, since the $\delta^{13}\text{C}$ ratio of soil organic matter had hardly changed (<1‰ difference between both experiments; Table S1). After the previous experiment, soil was homogenized within each AMF-nutrient combination two months before its start. Lime was added again to increase the pH on average to 6.19 (± 0.34). The soil of the same mesocosms as the previous experiment was pasteurized again by heating it for four hours at 80 °C to create ten AMF-free mesocosms. While N was sufficiently present and N fertilization in all mesocosms ensured no N limitations (see Verlinden *et al.*, 2018), we created a P-gradient by adding 2.5, 5, 10 or 20 kg P ha⁻¹ (triple superphosphate; Janssens-Smeets®) to soil of 20 mesocosms containing unpasteurized soil (further referred to as P1, P2, P3 and P4 treatments, respectively; 5 replicates per treatment). The 10 pasteurized mesocosms received either the same amount of P as the P1 (P1_noAMF) or the P4 (P4_noAMF) treatments (also 5 replicates per treatment). Fourteen seedlings of *Zea mays* L. (a C₄-plant, variety ‘Tom Thumb’) per mesocosm were planted on the 21th of June 2017 after their roots were submerged in spore-based inoculum of the AMF *Rhizophagus irregularis* (Symplanta®). Mesh bags, often used to study AMF (see e.g. Ven *et al.*, 2020) because they exclude ingrowth of roots but are permeable for fungal hyphae (30 µm mesh, size 13 cm × 3 cm × 2 cm, average soil density 1.497 g cm⁻³), filled with C free white river sand (heated for 5 hours at 550 °C to remove any remains of organic matter) were buried vertically into the top soil of each mesocosm one week before planting. One mesh bag per mesocosms was harvested at the end of the experiment, which was 10 weeks after planting.

Measurements

Environmental variables

In each mesocosm, soil water content was continuously measured using a reflectometer (CS616, Campbell Scientific, Logan, Utah, USA). To avoid any water stress, we ensured that soil water content (SWC) was always between 6% and 12%. Air temperature was logged simultaneously from a centralized air thermometer in the greenhouse. Two soil temperature sensors (NTC-WH probe, Carel Industries, Mannheim, USA) were installed in each mesocosm; soil temperature (°C) at 5 cm depth was logged every 15 minutes for each mesocosm. Photosynthetic photon flux density (PPFD) was continuously logged from PPFD sensors (JYP 1000, SDEC, Reignac-sur-Indre, France).

CO₂ flux measurements

An overview of the mesocosm measurements and their calculations to estimate C assimilation and partitioning can be found in Fig. 1. Mesocosm photosynthesis and respiration were quantified by chamber measurements of CO₂ fluxes, for which two cuvettes (one of 0.5 m height and one of 1.5 m height) were cross-calibrated at the start of the experiment (Fig. S1). During the experiment, CO₂-efflux increased and was always lower than 1.8 μmol CO₂ m⁻² s⁻¹ when the 0.5 m high cuvette was used while 1.85 μmol CO₂ m⁻² s⁻¹ was the lowest CO₂ flux measured using the 1.5 m high cuvette. These measurements were conducted ten times during the season (i.e. every week) for the inoculated mesocosms, and seven times during the season for the pasteurized mesocosms. Depending on plant height, one of the cuvettes was coupled to an EGM-4 infrared gas analyzer (PP Systems, Hitchin, UK) and used to cover the entire mesocosm to measure the CO₂ flux, operating as a dynamic closed system (De Boeck *et al.*, 2007; Vicca *et al.*, 2007; Verlinden *et al.*, 2018). Net ecosystem (mesocosm) exchange (NEE) of CO₂ was estimated from three to six measurements of 200 seconds per mesocosm at various light intensities (as in e.g. Vicca *et al.*, 2007 and Verlinden *et al.*, 2018) between 0 and 1300

$\mu\text{mol m}^{-2} \text{ s}^{-1}$ of PPFD using cloths of different transparency covering the mesocosm, attenuating incoming light. The CO_2 effluxes in a fully darkened cuvette represented total mesocosm CO_2 efflux (R_{eco}). GPP was then estimated by summing NEE and R_{eco} . Soil CO_2 efflux was measured weekly on a shallow inox collar (8 cm high, 7.5 cm $\varnothing$) which had been installed at 4 cm depth in each mesocosm prior to planting. Measurements were made using a custom-built cuvette (7.5 cm $\varnothing$, 16 cm height) coupled to an EGM-4 infrared gas analyser (PP Systems, Hitchin, UK) operating as a dynamic closed system. We measured the increase in soil CO_2 concentration on this shallow collar (R_{soil}) during 120 seconds. Additional to these measurements, from the fourth week until the end of the experiment, soil CO_2 efflux and its $\delta^{13}\text{C}$ signal were measured weekly (inoculated mesocosms) or every two weeks (pasteurized mesocosms) on the shallow collar and on an additional mesocosm-deep collar (60 cm high, 7.5 cm $\varnothing$). This collar covered the entire soil depth and thus prevented roots and fungi from growing in. Soil moisture inside these collars was measured every two weeks, and kept at field capacity, similar to the surrounding soil. The CO_2 efflux and the $\delta^{13}\text{C}$ signal were measured using a custom-built cuvette (7.5 cm $\varnothing$, 16 cm height) and a G2131-i CRDS isotopic- CO_2 gas analyser (Picarro Inc., Santa Clara, CA, USA) operating as a dynamic closed system. We targeted an increase of the CO_2 concentration inside the cuvette of at least 100 ppm which required between 5 and 15 minutes. Given the naturally occurring difference in $\delta^{13}\text{C}$ between the C_4 plants and the C_3 soil (in this experiment on average -12.25 in roots and -25.6 in soil; Table S1), these $\delta^{13}\text{C}$ flux measurements allowed distinguishing plant-derived C from C originating from the soil organic matter (see eq. S3 in Appendix S4, where we also refer to Table S2 and Fig. S2). As a complement to the mesocosm-scale chamber measurements, respiration and photosynthesis were measured also at leaf scale (Table S3). A portable LI-COR gas exchange system LI-6400 (Biosciences, Lincoln, NE, USA) was used, operating as an open system. Net

CO₂ assimilation rate was measured at 26 °C and photosynthetic photon flux densities (PPFD) of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (considered as maximal assimilation rate; A_{max}) and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (net assimilation at zero light, considered as ‘respiration in the dark’ (R_{dark})). The ratio $R_{\text{dark}}:A_{\text{max}}$ served as an indication of the fraction of photosynthates used in plant respiration.

For GPP, R_{eco} and R_{soil} we followed the same protocol as Verlinden *et al.* (2018); see also Halbritter *et al.* (2019). For each treatment, we fitted light response curves for GPP and temperature response curves for R_{eco} and R_{soil} . These were upscaled to the entire growing season to obtain a mean seasonal value and standard error (SE) for GPP (ΣGPP), R_{eco} (ΣR_{eco}) and R_{soil} (ΣR_{soil}) (we refer to Appendix S2, Appendix S3, and Tables S4, S5, S6, S7, S8 and S9 for more details regarding these calculations). The mean seasonal aboveground respiration (ΣR_{above}) was estimated as $\Sigma R_{\text{eco}} - \Sigma R_{\text{soil}}$. SE of the mean was calculated by error propagation.

As previously described in Ven *et al.* (2019), we calculated rhizosphere respiration (R_{rhizo}) using the Picarro CO₂ effluxes and associated $\delta^{13}\text{C}$ from the two collars installed in each mesocosm, to estimate total R_{rhizo} (ΣR_{rhizo}) over the entire growing season (see Appendix S4). SE of ΣR_{rhizo} was calculated by error propagation.

Plant biomass measurements and plant tissue P concentration

On 31 August 2017, when plant growth had stopped and corn cobs had ripened, aboveground and belowground plant biomass were harvested for all treatments, except for P1_noAMF where they had been harvested 9 days earlier because plants had died prematurely. All aboveground plant biomass was harvested in each mesocosm; belowground plant biomass was sampled taking six cores (7 cm $\varnothing$, entire soil depth). To approach a spatially representative estimate of belowground plant biomass production, two cores were collected on a stubble and the remainder at variable distances between the stubbles. Soil cores were sieved and roots were washed manually. Biomass was oven dried for 72 h at 70 °C. Subsamples were ground for further analyses.

For each mesocosm, the $\delta^{13}\text{C}$ of the roots was determined on a 2 mg subsample (Elemental Analysis – Isotope Ratio Mass Spectrometry (EA-IRMS) using a CE Instrument EA 1110 elemental analyser, coupled to a Finnigan MAT DeltaPlus IRMS with a Finnigan MAT ConFlo II Interface). The C concentration of different plant parts (roots, leaves, stems, cobs) was determined using an elemental analyser (Flash 2000, Thermo Fisher Scientific, Germany). P concentration (g P g^{-1} dry biomass) of each plant part was analysed after acid destruction using H_2SO_4 , salicylic acid, H_2O_2 and selenium (Walinga *et al.*, 1989). For each mesocosm, total plant biomass production (BP_{tot}) and P contents (P_{tot}) were calculated multiplying the biomass with C and P concentrations of the different plant parts. For each mesocosm, aboveground plant biomass production (BP_{above}) and belowground plant biomass production (BP_{root}) were calculated from the biomass and plant part-specific C concentrations. For each mesocosm, BPE was estimated as the fraction of BP_{tot} to the average $\sum\text{GPP}$ of its treatment.

C budget imbalance

To assess the robustness of our C partitioning estimates, we verified for each treatment the closure of the C balance, with $\sum\text{GPP}$ equalling the total C input, and the sum of $\sum\text{R}_{\text{aut}}$ and BP_{tot} (averaged per treatment) equalling total C output:

$$\text{C budget imbalance} = \text{total C input} - \text{total C output} = \sum\text{GPP} - (\sum\text{R}_{\text{aut}} + \text{BP}_{\text{tot}}) \quad (\text{eq. 1})$$

The relative C budget imbalance was calculated as:

$$\text{relative C budget imbalance} = \text{C budget imbalance} : \sum\text{GPP} \quad (\text{eq. 2})$$

SE was calculated using error propagation.

In our experiment, we did not explicitly include C partitioning to root exudation and mycorrhizal biomass. However, these C fluxes are indirectly included in $\sum\text{R}_{\text{aut}}$, since we assume turnover of both is fast (Olsson & Johnson, 2005; Phillips *et al.*, 2011; Ekblad *et al.*, 2016), and both are thus largely captured by R_{rhizo} (as part of R_{aut}).

Mycorrhizal colonization

Mycorrhizal root colonization and hyphal length followed the procedure described by Ven *et al.* (2020). Mycorrhizal root colonization was verified in August by sampling roots from four plants per mesocosm. Per plant, 20 cm of one lateral root containing root hair, was excavated, cut and stored for maximal two days at 5 °C. These roots were cleared and stained using a non-vital staining technique (as described by Vierheilig *et al.*, 2005), using a 5% KOH-solution and a Sheaffer Black Ink solution (10% ink in a 10% acetic acid solution). Mycorrhizal root colonization was quantified by counting arbuscules, vesicles and hyphae applying the gridline intersection method (Giovannetti and Mosse 1980, Brundrett *et al.*, 1996, Vierheilig *et al.*, 2005).

Hyphae were extracted from 4 g mesh bag sand following the approach of Rillig *et al.* (1999). See Appendix S5 for details. Hyphal intersects were counted at 400 × magnification at 25 locations on the filter using a grid inside the microscope ocular. Hyphal length density (HLD) was calculated using equation 3 (Rillig *et al.*, 1999; Tennant, 1975):

$$\text{HLD} = (\pi \cdot N \cdot A \cdot D) (H \cdot W)^{-1}, \quad (\text{eq. 3})$$

with HLD = hyphal length density (mm hyphae g⁻¹ soil), N = number of intersects where AMF hyphae were present, A = area of filter (mm²) that was examined, D = dilution factor, H = total length of raster lines projected on filter (mm), and W = soil weight (g).

Statistical analysis

Statistical analyses were performed in R (RStudio Team, 2016). We had two types of variables: 1) ‘Data_type1’, was comprised of variables that were estimated for each mesocosm separately: BP_{root}, BP_{above}, BP_{tot}, R_{dark}:A_{max}, mycorrhizal colonization in roots, and HLD. Also BP_{root}:ΣGPP, BP_{above}:ΣGPP, and BPE (i.e. BP_{tot}:ΣGPP) were calculated for each mesocosm separately (but using the treatment ΣGPP value; see data_type2); 2) ‘Data_type2’, were variables with only one value per treatment. These combined data from all mesocosms of a treatment to enable robust fitting of temperature or light response curves : ΣGPP, ΣR_{rhizo},

261 $\sum R_{\text{above}}$, $\sum R_{\text{aut}}$, $\sum R_{\text{rhizo}}$: $\sum \text{GPP}$, $\sum R_{\text{above}}$: $\sum \text{GPP}$, $\sum R_{\text{aut}}$: $\sum \text{GPP}$. Given that $\sum \text{GPP}$ and $\sum R_{\text{aut}}$ were
 262 data_type2, also the C budget imbalance and relative C budget imbalance were data_type2.
 263 BP_{tot} was averaged per treatment for these calculations (i.e., converted from data_type1 to
 264 data_type2; see eq. 1). Data analyses differed between both types in that data_type2 represented
 265 only a summary value with a standard error, calculated using error propagation. Therefore,
 266 variables belonging to data_type2 were analysed with a weight variable, inversely proportional
 267 to the standard error. This approach does not allow calculation of the correlation coefficient
 268 (R^2), which is thus not presented for data_type2.
 269 For each treatment a 95% confidence interval was calculated as $1.96 \times \text{SE}$ to check if the C
 270 budget imbalance differed from zero. A linear model was fitted to test the correlation between
 271 P fertilization level (2.5, 5, 10 and 20 kg P ha⁻¹) and data_type1 in inoculated mesocosms. This
 272 linear model was also fitted for data_type2.
 273 Additionally, a one-way analysis of variance (ANOVA) was carried out for inoculated
 274 mesocosms to test if data_type1 differed between 2.5, 5, 10 and 20 kg ha⁻¹ as four independent
 275 treatments: 'P1', 'P2', 'P3' and 'P4' respectively. Pairwise differences between these treatments
 276 were estimated using a posthoc analysis with Tukey's Honest Significant Difference
 277 (TukeyHSD) method for multiple hypothesis testing. For $\sum \text{GPP}$, which in inoculated
 278 mesocosms could be estimated for each of the nine measuring periods separately, pairwise
 279 differences between the four treatments were calculated using R-package 'multcomp' and its
 280 Simultaneous Tests for General Linear Hypotheses (SGLH) with the 'Tukey Contrasts'
 281 method, with measuring period as independent variable and the inverse of the SE of each
 282 treatment in every nine measurement periods added as weight. In other data_type2 datasets,
 283 where only one average value per treatment over all measurement periods could be calculated,
 284 significance of differences between two treatments was tested by directly filling in the test

statistic of the t-test, with differences between the two means in the numerator, and the common standard error (calculated by error propagation) in the denominator.

A t-test using only the pasteurized ‘P4_noAMF’ and inoculated soil ‘P4’ treatment was applied to test the pasteurization effect on data_type1 datasets. For $\sum GPP$, this pasteurization effect was calculated using R-package ‘multcomp’ and its SGLH with the ‘Dunnett Contrasts’ method, with measuring period as independent variable and weighting the observations by the inverse of the standard error. In the other data_type2 datasets, the significance of the pasteurization effect was estimated as described above, directly filling out the difference between P4 and P4_noAMF in the numerator of the test statistic for the t-test, and divided by their common SE (calculated using error propagation).

RESULTS

For all treatments, the C budget imbalance was relatively small, and for treatments P1, P2, P3 and P4_noAMF, this was not significantly different from zero (Table 1). Only in treatment P4, the ‘C budget imbalance’ was significantly negative (Table 1). Fertilization or pasteurization did not significantly affect the C budget imbalance (Table 1). The relative C budget imbalance (i.e., relative to $\sum GPP$) varied between -0.06 and 0.01 for all treatments and did not significantly differ among treatments (Table 2). Pasteurization had no effect on this relative C budget imbalance (Table 2).

$\sum GPP$, $\sum R_{rhizo}$, $\sum R_{above}$, $\sum R_{aut}$ (the sum of $\sum R_{rhizo}$ and $\sum R_{above}$), BP_{root} , BP_{above} and BP_{tot} (the sum of BP_{root} and BP_{above}) all increased or tended to increase with increasing P fertilization (Table 1), while pasteurization (P4 vs. P4_noAMF) significantly decreased all these estimates of plant growth and activity (Table 1). Both $\sum R_{above}:\sum GPP$ and $R_{dark}:A_{max}$ increased with increasing P fertilization ($p \leq 0.06$). $\sum R_{rhizo}:\sum GPP$, on the other hand, was not affected by P fertilization (Table 2). $\sum R_{aut}:\sum GPP$ did significantly increase with increasing P fertilization

(Fig. 2). Pasteurization had a significantly positive effect on $\sum R_{\text{above}}:\sum GPP$, but a significantly negative effect on $\sum R_{\text{rhizo}}:\sum GPP$, and no significant effect on $R_{\text{dark}}:A_{\text{max}}$ or $\sum R_{\text{aut}}:\sum GPP$ (Table 2, Fig. 2).

The relationships for $BP_{\text{above}}:\sum GPP$ and $BP_{\text{root}}:\sum GPP$ versus P fertilization were not statistically significant. Nonetheless, $BP_{\text{root}}:\sum GPP$ was twice as large in P1 and P2 as compared to P3 and P4, with a significant difference between P1 and P2 vs P3 and P4 (Table 2). Also BPE tended to decrease with P fertilization and ANOVA showed a significant difference between treatments: both P4 and P3 < P2 (both $p = 0.01$). Pasteurization did not affect $BP_{\text{root}}:\sum GPP$, $BP_{\text{above}}:\sum GPP$ or BPE (Table 2, Fig. 2).

Mycorrhizal colonization in roots significantly increased with P fertilization when expressed as % of roots colonized by arbuscules (on average $\pm 10\%$ in P1, P2 and P3 vs. 22% in P4), had a trend towards a significant increase with P fertilization when expressed as % of roots colonized by hyphae (on average ± 35 to 60%), and did not increase with P fertilization when expressed as % of roots colonized by vesicles (on average approx. 10 to 30 %; Table 3). Despite substantial root colonization (Table 3), HLD in soil was low (Fig. 3; HLD 50 % lower than in Verlinden *et al.*, 2018). P fertilization significantly increased the hyphal length density (HLD), although this correlation was rather weak (after $\ln$ -transformation, Fig. 3A). In the pasteurized mesocosms, no mycorrhizal structures were found in roots or soil (Table 3, Fig. 3).

DISCUSSION

To verify the robustness of our estimation of C partitioning to R_{aut} and to plant growth, we confirmed the closure of the C balance; a closed C balance indicates robust estimation of partitioning. This was the case for all treatments except for P4, where the C budget imbalance indicated an underestimation of C input ($\sum GPP$) and/or overestimation of C outputs ($\sum R_{\text{aut}}$ and/or $\sum BP_{\text{tot}}$; see eq. 1). Importantly, the relative C budget imbalance was small for all

treatments (no more than 6% of assimilated C) and was not significantly affected by P
 fertilization. We can thus assume that the observed fertilization effects on C partitioning were
 not biased by the methodological challenge to close the C balance. In our calculations, we
 further assume that C partitioning to root exudation and to AMF was largely captured by R_{rhizo} ,
 because turnover of both is fast (Olsson & Johnson, 2005; Phillips *et al.*, 2011; Ekblad *et al.*,
 2016). The pasteurized treatment, where the absence of AMF was expected to facilitate the C
 balance-closure, allowed us to verify this assumption. The lack of a negative pasteurization
 effect on the (relative) C budget imbalance of the P4 treatment confirms that C partitioning to
 root exudation and mycorrhizal symbiosis was largely captured by R_{rhizo} .
 Increasing P fertilization clearly increased $\sum \text{GPP}$, $\sum R_{\text{aut}}$ and BP, indicating that P availability
 limited plant growth and activity in the lower P treatments. The largest increase was observed
 for $\sum \text{GPP}$, indicating that $\sum \text{GPP}$ is more sensitive to changes in P availability than BP. This
 contrasted with our expectation that plant growth would be more responsive to variation in
 nutrient availability than photosynthesis (e.g. Vicca *et al.*, 2012; Verlinden *et al.*, 2018). The P
 limitation was also reflected in the P-induced decrease of $\text{BP}_{\text{root}}:\sum \text{GPP}$, which was twice as
 large in P1 and P2 as compared to P3 and P4. Increasing nutrient availability often reduces the
 need of plants to invest C belowground for acquiring nutrients, leaving more C for aboveground
 growth (Lilleskov *et al.*, 2002; Treseder, 2004; Vicca *et al.*, 2012). Surprisingly, the reduction
 in $\text{BP}_{\text{root}}:\sum \text{GPP}$ with P fertilization in our experiment was not associated with an increase in
 $\text{BP}_{\text{above}}:\sum \text{GPP}$, and C partitioning to BP_{tot} , i.e. BPE, even tended to decrease with increasing P
 fertilization. This contrasts with our hypothesis and with earlier research (e.g. Litton *et al.*,
 2007; Vicca *et al.*, 2012; Verlinden *et al.*, 2018). The tendency for a decrease instead of an
 increase of BPE with increasing P fertilization implies that P fertilization either increased C
 partitioning to plant respiration, increased C partitioning to symbionts and exudates, or a
 combination of both.

360 C partitioning to autotrophic respiration ($\sum R_{\text{aut}}:\sum \text{GPP}$) indeed increased with increasing P
 361 fertilization. However, $\sum R_{\text{aut}}$ includes $\sum R_{\text{rhizo}}$ which at least partly captured symbionts and
 362 exudates in our calculations. Distinguishing above- versus belowground patterns provides
 363 further insights in the underlying processes. Both $\sum R_{\text{above}}:\sum \text{GPP}$ and the leaf respiration-to-
 364 photosynthesis ratio ($R_{\text{dark}}:A_{\text{max}}$; see Table S3) increased with increasing P fertilization. This
 365 supports studies that indicated that the respiration-to-photosynthesis ratio may be less constant
 366 than previously assumed, especially under less favourable growth conditions (Atkin *et al.*,
 367 2007; Campbell *et al.*, 2007; Drake *et al.*, 2016; Collalti & Prentice, 2019). In contrast to these
 368 aboveground patterns, $\sum R_{\text{rhizo}}:\sum \text{GPP}$ was unaffected by P fertilization. We can thus conclude
 369 that the decreasing trend in BPE with increasing P fertilization was primarily linked to
 370 increased C partitioning to plant respiration, especially aboveground. Possibly, mass-specific
 371 leaf respiration increased with increasing P due to increased labile carbon following low sink
 372 demand (Mahmud *et al.*, 2018). Such effect may be more likely to occur when AMF abundance
 373 is low (Valentine *et al.*, 2001), as was the case in our experiment, but we do not have the data
 374 to verify whether sink limitation occurred in our experiment.

375 The relatively constant $\sum R_{\text{rhizo}}:\sum \text{GPP}$ contrasted with the reduction of $\text{BP}_{\text{root}}:\sum \text{GPP}$ with
 376 increasing P fertilization. Hence, the reduced C partitioning to root biomass production
 377 appeared to be counterbalanced by C partitioning to other belowground processes, such as
 378 mycorrhizal symbiosis, exudation and/or root respiration, as reflected also in the increase of
 379 $\sum R_{\text{rhizo}}:\text{BP}_{\text{root}}$ with increasing P (Table S10). Although AMF abundance was low, root
 380 mycorrhizal colonization and HLD (a proxy for AMF biomass) increased with increasing P
 381 availability and the possibility for an increased contribution of AMF to $\sum R_{\text{rhizo}}:\text{GPP}$ with
 382 increasing P fertilization is supported by the difference between inoculated and pasteurized
 383 mesocosms. Both $\sum R_{\text{rhizo}}:\sum \text{GPP}$ and $\sum R_{\text{rhizo}}:\text{BP}_{\text{root}}$ were smaller for the pasteurized treatment
 384 (Tables 2 and S10). On the other hand, we cannot exclude the possibility that C partitioning to

385 exudation and/or root respiration contributed to the increasing $\sum R_{\text{rhizo}}:BP_{\text{root}}$ with increasing P.
 386 Our data do not allow to further unravel the contribution of these processes; in future
 387 experiments, measurements of specific root respiration can provide further insight into these
 388 mechanisms.

389 The unexpected decreasing trend for BPE in response to P fertilization contrasted with the P-
 390 induced increase of BPE observed in a similar experiment conducted one year earlier
 391 (Verlinden *et al.*, 2018; Fig. 2). In this previous experiment, the same set-up, soil and plant
 392 species were used, but fertilizer treatments differed (full factorial N×P experiment; see methods
 393 and Verlinden *et al.*, 2018). Given that the mesocosms of P1 and P4 received comparable
 394 amounts of P in both experiments, and N fertilization did not affect plant growth or C
 395 partitioning in the previous experiment, the contrasting response of BPE to P fertilization is
 396 unlikely due to differences in fertilizer treatments between both experiments. Even though
 397 $\sum R_{\text{aut}}:\sum GPP$ could only be estimated indirectly in the previous experiment (i.e., as the residual
 398 C needed to close the C balance), also these patterns clearly differed between both experiments:
 399 both $\sum R_{\text{aut}}:\sum GPP$ and $R_{\text{dark}}:A_{\text{max}}$ increased with P fertilization in the current experiment but
 400 were relatively constant in the previous experiment. These differences between both
 401 experiments emphasize a fundamental difference in the C partitioning process.

402 A remarkable difference between both experiments was the degree of AMF abundance in soil;
 403 the amount of AMF was much lower in the current than in the previous experiment (e.g., HLD
 404 in the current experiment was only about half the amount of the previous experiment; Fig. 3).
 405 This may indicate a role for the AMF in explaining differences in C partitioning between both
 406 experiments. This is further supported by the fact that the differences in BPE and $\sum R_{\text{aut}}:\sum GPP$
 407 between both experiments were especially clear for their lowest fertilization level (Fig. 2),
 408 where plants relied most on their symbionts for nutrient uptake. Combined, the two
 409 experiments suggest that, when AMF abundance is high (exp. 1), plants growing on P poor soil

invest more of their photosynthates in these symbiotic partners, at the expense of C partitioning to BP, hence resulting in an increase of BPE with increasing P fertilization. When AMF abundance was low (exp. 2 and pasteurized treatment), possibly due to unfavourable soil conditions, plant C partitioning responded very different, with no increase in C partitioning to plant biomass, but increased partitioning to R_{aut} . Further experimental testing is needed to verify this postulated role for AMF in plant C partitioning and to unravel the physiology behind these differences.

A possible reason for the lower AMF growth in the current experiment is a reduction in soil pH. Due to somewhat different liming treatments, the average soil pH declined from 6.19 to 5.43 during the current experiment (Table S11), while during the previous experiment soil pH increased from 6.84 to 7.44 (Table S11). AMF typically thrive in high pH soils, (Marschner, 1998; Clark, 2002; van Aarle *et al.*, 2002), and at high pH, P is increasingly locked up as calcium phosphate, making plants more dependent on AMF for P acquisition (Larcher, 2003). Despite their lower abundance in soil, AMF still seemed to have a positive effect on plant performance in the current experiment. BP_{tot} and ΣGPP were about 50% higher in the inoculated than in the pasteurized P4 mesocosms (P4 vs. P4_noAMF) and plants in pasteurized P1 mesocosms (P1_noAMF) died prematurely while those in P1 survived the similarly low P addition. Also the lower N:P ratio in leaves of AMF-colonized plants in comparison with leaves of plants in pasteurized mesocosms indicate that plants were more P-stressed in the pasteurized mesocosms (Table S12).

In summary, while previous research reported a decrease in BPE with decreasing nutrient availability (Vicca *et al.*, 2012), our experiment unveiled a more complex picture. The main hypothesis that BPE increases with increasing P fertilization was refuted, and while $R_{\text{aut}}:GPP$ was expected to be relatively constant among treatments, we found an increase with increasing P fertilization due to increased C partitioning to aboveground respiration. The difference

between pasteurized (no AMF) and AMF-inoculated treatments, together with the comparison with a previous experiment in a similar set-up, suggest a role for AMF in determining these C partitioning patterns; as suggested by Ven et al. (2019), AMF can reduce the C cost of P uptake, even when their abundance and hence their C use is low. Our results emphasize the need to take into account not only nutrient availability, but also mycorrhizal symbionts when studying and modelling C partitioning in terrestrial ecosystems.

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AUTHOR CONTRIBUTION

SV designed the research; AV and MV conducted the field- and lab work; AV drafted the paper; and AV, MV, EF, PAO, EV, HW and SV contributed to the interpretation of the results and to the writing of the paper, where EF and AV made statistical decisions and calculations.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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TABLES

Table 1: Mean and standard error (SE) of total gross primary production (ΣGPP), total rhizosphere respiration (ΣR_{rhizo}), total aboveground respiration (ΣR_{above}), total autotrophic respiration (ΣR_{aut}), belowground plant biomass production (BP_{root}), aboveground plant biomass production (BP_{above}), plant biomass production (BP_{tot}), and the difference between plant C input (ΣGPP) and plant C output ($\Sigma R_{aut} + \Sigma BP_{tot}$) (C budget imbalance), in $g\ C\ m^{-2}$, for each treatment (P1, P2, P3, P4, P1_noAMF and P4_noAMF). p values and correlation coefficient (R^2) from a linear regression indicate the strength of the relationships with P fertilization ($kg\ P\ ha^{-1}$). p values from one-way ANOVA show pasteurization effects for P4_noAMF versus P4. Subscript letters indicate internal differences between P1, P2, P3 and P4 as results from post-hoc analysis of one-way ANOVA; 95% confidence interval (conf. int.) for C budget imbalance was given.

treatment	ΣGPP		ΣR_{rhizo}		ΣR_{above}		ΣR_{aut}		BP_{root}		BP_{above}		BP_{tot}		C budget imbalance		
	$g\ C\ m^{-2}$		$g\ C\ m^{-2}$		$g\ C\ m^{-2}$		$g\ C\ m^{-2}$		$g\ C\ m^{-2}$		$g\ C\ m^{-2}$		$g\ C\ m^{-2}$		$g\ C\ m^{-2}$		
	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	95% conf. int.
P1	101.42 ^a	5.76	11.21 ^a	1.64	41.53 ^a	6.51	52.74 ^a	6.71	10.60 ^a	1.92	37.69 ^a	3.83	48.29 ^a	4.53	0.39 ^a	9.83	-18.88 ~ 19.66
P2	116.86 ^a	5.57	15.53 ^a	2.32	50.46 ^a	6.58	65.99 ^a	6.98	14.07 ^{ab}	1.28	41.05 ^a	1.33	55.11 ^a	2.23	-4.25 ^a	9.12	-22.13 ~ 13.63
P3	435.37 ^b	6.93	46.34 ^b	6.64	219.63 ^b	7.60	265.97 ^b	10.09	20.87 ^{bc}	3.43	148.20 ^b	9.66	169.07 ^b	11.52	0.34 ^a	15.97	-30.96 ~ 31.64
P4	497.70 ^b	7.48	67.15 ^c	7.29	257.05 ^c	7.22	324.20 ^c	10.26	23.25 ^c	2.31	179.15 ^c	6.46	202.40 ^c	7.67	-28.89 ^a	14.43	-0.61 ~ -57.17***
P1_noAMF	10.51	NA**	3.21	0.32	5.55	NA**	8.76	NA**	0.20	0.03	1.82	0.15	2.02	0.17	-0.27	NA**	NA**
P4_noAMF	256.10	9.69	15.02	1.37	162.61	8.88	177.62	8.98	13.12	1.06	79.78	7.39	92.90	7.50	-14.43	15.17	-44.16 ~ 15.30
Fertilization effect	p = 0.07		p = 0.03		*p = 0.06		*p = 0.06		*p = 0.01 R ² = 0.98		p = 0.06 R ² = 0.92		*p = 0.09 R ² = 0.95		p = 0.10 R ² = 0.89		
Pasteurization effect	p = 0.03		p < 0.01		p < 0.01		p < 0.01		p < 0.01		p < 0.01		p < 0.01		p = 0.49		

621 Notes: * significance after ln-transformation; **Data for P1_noAMF were not included in statistical analyses or partitioning calculations, since
622 plants were too small for CO₂ measurements and died off prematurely; ***95% conf. int. indicates mean is significantly different from zero

Table 2: Mean and standard error (SE) of total rhizosphere respiration (ΣR_{rhizo}), total aboveground respiration (R_{above}), belowground plant biomass production (BP_{root}), and aboveground plant biomass production (BP_{above}) relative to gross primary production (GPP), the difference between ΣGPP and $\Sigma R_{aut} + \Sigma BP_{tot}$ relative to ΣGPP (relative C budget imbalance), and leaf level dark respiration relative to maximum leaf CO_2 assimilation ($R_{dark}:A_{max}$) for each treatment (P1, P2, P3, P4 and P4_noAMF). p values and correlation coefficient (R^2) from a linear regression indicate the strength of the relationships with P fertilization ($kg\ P\ ha^{-1}$). p values from a one-way ANOVA show pasteurization effects for P4_noAMF versus P4. Subscript letters indicate internal differences between P1, P2, P3 and P4 as results from post-hoc analysis of one-way ANOVA.

treatment	$\Sigma R_{rhizo}:\Sigma GPP$		$\Sigma R_{above}:\Sigma GPP$		$R_{dark}:A_{max}$		$BP_{root}:\Sigma GPP$		$BP_{above}:\Sigma GPP$		relative C budget imbalance	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
P1	0.11 ^a	0.02	0.41 ^a	0.07	0.0640 ^a	0.0051	0.10 ^a	0.02	0.37 ^a	0.04	< 0.01 ^a	0.10
P2	0.13 ^a	0.02	0.43 ^{ab}	0.06	0.0685 ^{ab}	0.0103	0.12 ^a	0.01	0.35 ^a	0.02	-0.04 ^a	-0.08
P3	0.11 ^a	0.02	0.50 ^{bc}	0.02	0.0931 ^b	0.0082	0.05 ^b	0.01	0.34 ^a	0.02	< 0.01 ^a	0.04
P4	0.13 ^a	0.01	0.52 ^c	0.02	0.0892 ^b	0.0071	0.05 ^b	0.005	0.36 ^a	0.01	-0.06 ^a	-0.03
P4_noAMF	0.06	0.01	0.63	0.04	0.0767	0.0078	0.05	0.005	0.31	0.03	-0.06	-0.06
Fertilization effect	p = 0.48		*p = 0.06		p = 0.05 R ² = 0.95		*p = 0.15 R ² = 0.83		p = 0.87 R ² = -0.18		p = 0.23	
Pasteurization effect	p < 0.01		p = 0.01		p = 0.95		p = 0.49		p = 0.16		p = 0.98	

*Note: * significance after ln-transformation*

Table 3: Mean and standard error (SE) of mycorrhizal colonization in roots, expressed as % of roots colonized by hyphae, arbuscules or vesicles for each treatment (P1, P2, P3, P4, P1_noAMF and P4_noAMF). p values and correlation coefficient (R^2) from a linear regression indicate the strength of the relationships with P fertilization (kg P ha^{-1}). Subscript letters indicate internal differences between P1, P2, P3 and P4 as results from post-hoc analysis of one-way ANOVA.

treatment	mycorrhizal colonization in roots					
	hyphae		arbuscules		vesicles	
	%		%		%	
	mean	se	mean	se	mean	se
P1	50.40 ^a	6.62	11.60 ^a	3.12	20.00 ^a	6.23
P2	35.60 ^a	11.07	7.20 ^a	3.72	11.60 ^a	5.53
P3	53.20 ^a	5.24	9.20 ^a	2.42	14.00 ^a	2.19
P4	63.73 ^a	9.03	22.13 ^a	5.81	28.47 ^a	6.98
P1_noAMF	0.00	0.00	0.00	0.00	0.00	0.00
P4_noAMF	0.00	0.00	0.00	0.00	0.00	0.00
Fertilization effect	p = 0.08 $R^2 = 0.40$		p = 0.03 $R^2 = 0.50$		p = 0.13 $R^2 = 0.35$	

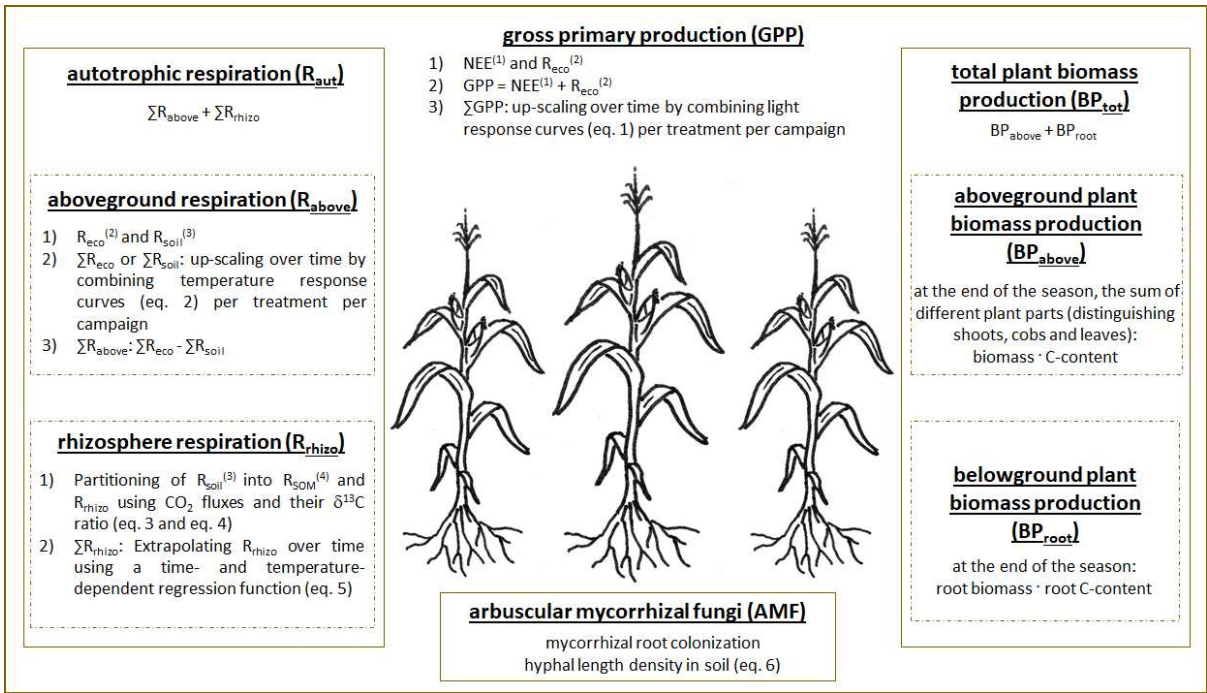


Figure 1: overview of the components of plant C partitioning as assessed in the experiment. Equations (eq. 1 to 6) refer to the calculations explained in the text (see ‘Measurements’).
Notes: $NEE^{(1)}$: net ecosystem exchange of CO_2 , estimated from mesocosm-scale measurements at various light intensities; $R_{eco}^{(2)}$: total ecosystem CO_2 efflux obtained by measurements in a fully darkened chamber; $R_{soil}^{(3)}$: soil respiration obtained by measurements on the shallow soil collar; $R_{SOM}^{(4)}$: soil organic matter decomposition CO_2 efflux obtained by measurements on the mesocosm-deep collar (i.e., no ingrowth of roots or AMF).

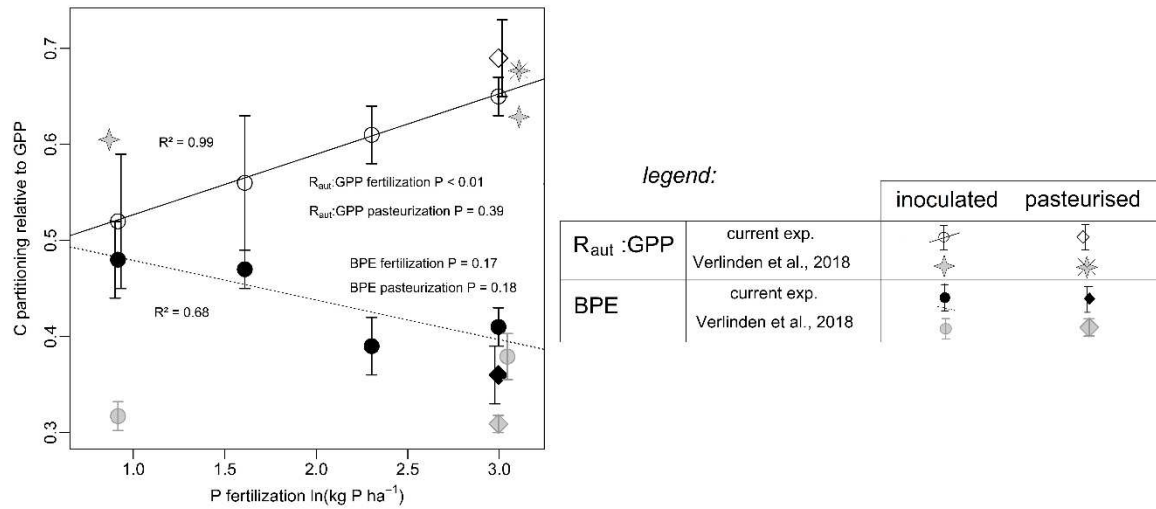
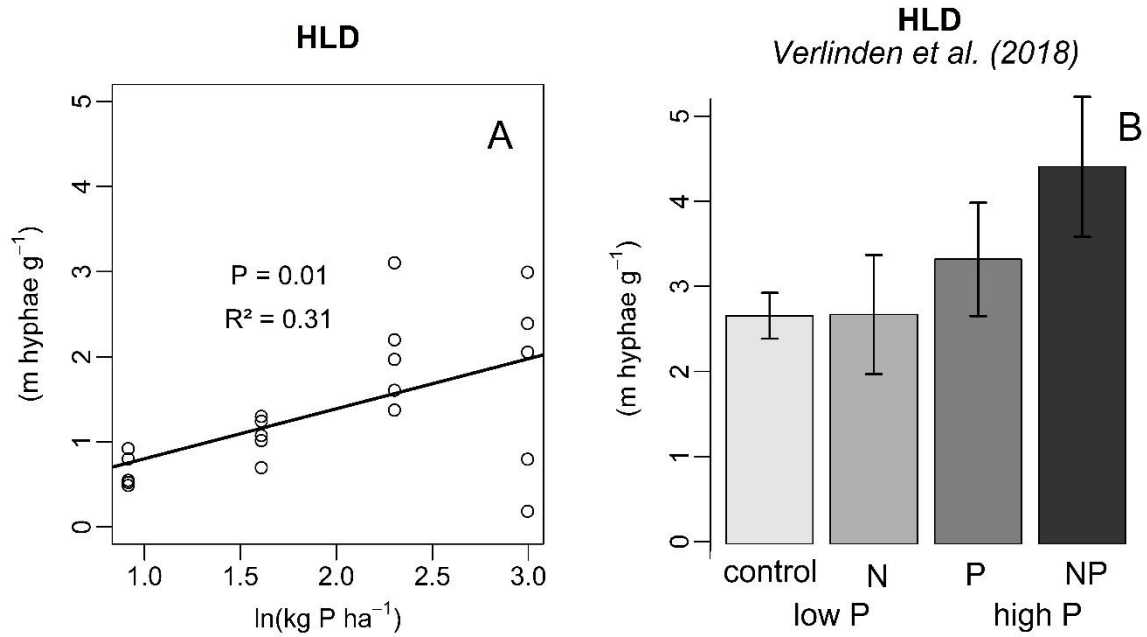


Figure 2: Average biomass production efficiency ($BPE = \text{total biomass production relative to photosynthesis } (\sum GPP)$ black), and $\sum R_{aut} : \sum GPP$ (total autotrophic respiration relative to $\sum GPP$, white) for each treatment (P fertilization 2.5, 5, 10 or 20 kg P ha^{-1}) in AMF inoculated mesocosms ($\circ$) and pasteurized $P4$ mesocosms ($P4_noAMF$, $\diamond$). p values are shown for a linear regression between BPE or $\sum R_{aut} : \sum GPP$ versus P fertilization (kg P ha^{-1}). A full line indicates statistical significant, a dotted line indicates that the regression was not statistically significant. p values for the pasteurization effect are obtained from a one-way ANOVA comparing $P4_noAMF$ to $P4$. Error bars show ± 1 standard error (SE). BPE and $\sum R_{aut} : \sum GPP$ of comparable treatments from Verlinden et al. (2018) are also shown (grey circles and grey stars, respectively).



668

669 Figure 3: hyphal length density (HLD; m hyphae g⁻¹ sand) in mesh bags for each treatment (P

670 fertilization; log(kg P ha⁻¹)) in AMF inoculated mesocosms (A). p values and correlation

671 coefficient (R²) from a linear regression indicate the strength of the relationships with P

672 fertilization (kg P ha⁻¹) after ln-transformation. Average and SE of HLD from Verlinden et al.

673 (2018) are shown in panel (B).

SUPPORTING INFORMATION LEGENDS

Appendix S1: experimental set up and soil mixture, as in Verlinden et al. (2018).

Appendix S2: CO₂ fluxes data analyses for mean seasonal values and standard errors (SE) for GPP, R_{eco} and R_{soil} .

Appendix S3: the use of T_{soil} instead of T_{air} for calculating and upscaling R_{eco} .

Appendix S4: Rhizosphere respiration (R_{rhizo}) calculations and estimation of total R_{rhizo} ($\sum R_{rhizo}$) over the entire growing season, as in Ven et al. (2019).

Appendix S5: Hyphal extraction from mesh bag sand following the approach of Rillig et al. (1999).

Table S1: mean and standard error (SE) of soil organic matter (SOM) % C and $\delta^{13}C$ ratio, and root $\delta^{13}C$ ratio at the start of the previous experiment (Verlinden et al., 2018) and the current experiment.

Table S2: mean, standard error (SE) and correlation coefficient after statistical analysis (r) of cumulated rhizosphere respiration ($\sum R_{rhizo}$) ($\mu\text{mol C m}^{-2} \text{ s}^{-1}$) calculated with and without adding an intercept for day 0, $R_{rhizo} = 0$ to the dataset.

Table S3: mean and standard error (SE) of dark respiration (R_{dark}), maximum leaf CO₂ assimilation (A_{max}) and their ratio ($R_{dark}:A_{max}$) for each treatment (P1, P2, P3, P4 and P4_noAMF).

699

700 *Table S4: R_{soil} as a percentage of R_{eco} for seven time periods, starting at day of planting (21/06)*
701 *and ending at plant harvest (31/08).*

702

703 *Table S5: R^2 of fits using soil temperature (T_{soil}) or air temperature (T_{air}) to calculate $\sum R_{eco}$,*
704 *and the difference between R^2 of these fits using T_{soil} and T_{air} , for each treatment in four time*
705 *periods during the current experiment. Mean, maximum (max) and minimum (min) R^2 over*
706 *these four time periods for each treatment are given.*

707

708 *Table S6: R^2 of fits using soil temperature (T_{soil}) or air temperature (T_{air}) to calculate $\sum R_{eco}$,*
709 *and the difference between R^2 of these fits using T_{soil} and T_{air} , for each treatment in seven time*
710 *periods during the experiment of Verlinden et al. (2018). Mean, maximum (max) and minimum*
711 *(min) R^2 over these seven time periods for each treatment are given.*

712

713 *Table S7: $\sum R_{eco}$ at the end of the experiment discussed in Verlinden et al. (2018), and their*
714 *difference and deviation (%) for each treatment. The mean deviation was calculated over all*
715 *treatments (in **bold**).*

716

717 *Table S8: Results for using soil temperature (T_{soil}) to calculate and upscale ecosystem*
718 *respiration (R_{eco}). Average (mean) and standard error (SE) of total gross primary production*
719 *($\sum GPP$), total aboveground respiration ($\sum R_{above}$), total autotrophic respiration ($\sum R_{aut}$), the*
720 *difference between plant C input ($\sum GPP$) and plant C output ($\sum R_{aut} + BP_{tot} = C$ budget*
721 *imbalance), in $g\ C\ m^{-2}$ and of $\sum R_{above}$, $\sum R_{aut}$ and C budget imbalance relative to gross primary*
722 *production (GPP), i.e. respectively $\sum R_{above}:\sum GPP$, $\sum R_{aut}:\sum GPP$ and the relative C budget*

imbalance for each treatment (P1, P2, P3, P4 and P4_noAMF). These results and their statistics can also be found in Tables 1, 2 and Fig. 2.

Table S9: Results for using air temperature (T_{air}) to calculate and upscale ecosystem respiration (R_{eco}). Average (mean) and standard error (SE) of total gross primary production (ΣGPP), total aboveground respiration (ΣR_{above}), total autotrophic respiration (ΣR_{aut}), the difference between plant C input (ΣGPP) and plant C output ($\Sigma R_{aut} + BP_{tot} = C$ budget imbalance), in $g\ C\ m^{-2}$ and of ΣR_{above} , ΣR_{aut} and C budget imbalance relative to gross primary production (GPP), i.e. respectively $\Sigma R_{above}:\Sigma GPP$, $\Sigma R_{aut}:\Sigma GPP$ and the relative C budget imbalance for each treatment (P1, P2, P3, P4 and P4_noAMF).

Table S10: mean, standard error (SE) and correlation coefficient after statistical analysis (R^2) of cumulated rhizosphere respiration (ΣR_{rhizo}) ($\mu mol\ C\ m^{-2}\ s^{-1}$) relative to belowground plant biomass production (BP_{root}) and aboveground plant biomass production (BP_{above}). p values from a linear regression show correlations with P fertilization; p values from one-way ANOVA show pasteurization effect between P4 and P4_noAMF.

Note: * significance after $\ln$ -transforming

Table S11: mean and standard error (between brackets) pH at the start, the middle and the end of the previous experiment (2016; published in Verlinden et al., 2018) and the current experiment (2017).

Table S12: mean and standard error (SE) of P and N concentration ($[P]$ and $[N]$), and its N:P ratio of leaves in different treatments in 2017 and 2016, for 2017. p value of ANOVA showed significance in differences due to fertilization or pasteurization (P4 vs. P4_noAMF).

748

749 *Figure S1: comparison of CO₂ flux measurement cuvettes.*

750

751 *Figure S2: observed (black circles) and predicted (grey triangles) rhizosphere respiration*
752 *(R_{rhizo}) ($\mu\text{mol C m}^{-2} \text{ s}^{-1}$) in time (days since planting) for each phosphorus (P) fertilization*
753 *treatment. Correlation coefficient (R^2) reflects the goodness of fit for observed vs. predicted.*