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Serendipita bescii promotes winter wheat growth and modulates the host root transcriptome under phosphorus and nitrogen starvation

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Summary

***Serendipita vermifera* ssp. *bescii*, hereafter referred to as *S. bescii*, is a root-associated fungus that promotes plant growth in both its native switchgrass host and a variety of monocots and dicots. Winter wheat (*Triticum aestivum* L.), a dual-purpose crop, used for both forage and grain production, significantly contributes to the agricultural economies of the Southern Great Plains, USA. In this study, we investigated the influence of *S. bescii* on growth and transcriptome regulation of nitrogen (N) and phosphorus (P) metabolism in winter wheat. *Serendipita bescii* significantly improved lateral root growth and forage biomass under a limited N or P regime. Further, *S. bescii* activated sets of host genes regulating N and P starvation responses. These genes include, root-specific auxin transport, strigolactone and gibberellin biosynthesis, degradation of phospholipids and biosynthesis of glycerolipid, downregulation of ammonium transport and nitrate assimilation, restriction of protein degradation by autophagy and subsequent N remobilization. All these genes are hypothesized to regulate acquisition, assimilation and remobilization of N and P. Based on transcriptional level gene regulation and physiological responses to N or P limitation, we suggest *S. bescii* plays a critical role in modulating stress imposed by limitation of these two critical nutrients in winter wheat.**

Introduction

Wheat (*Triticum aestivum* L.) is a cereal grown on more land area than any other commercial food crop. Hard red winter wheat is a dominant dual-purpose (forage and grain) crop in the Southern Great Plains (Kansas, Oklahoma, and Texas), with approximately 8 million ha planted every year. In the state of Oklahoma, roughly 2 million ha of winter wheat are cultivated annually accounting for 75% of the state's total cropland (Patrignani *et al*., 2014). NF101 is a hard red winter wheat cultivar developed by the Noble Research Institute, LLC and is a top performing dual-purpose cultivar in Oklahoma and North Texas.

Phosphorus (P) is an essential element for many fundamental processes in plant life, including photosynthesis, respiration, cellular membrane construction, and signal transduction, yet is limiting in most soils. Concentrations of P in the soil are typically 10 μM or less, and readily form sparingly soluble salts with Ca, Fe and Al (Pierzynski and McDowell, 2005). Wheat consumes much more P fertilizer than rice (*Oryza sativa* L.) and maize (*Zea mays* L.) every year (Yuan *et al*., 2016), and the average yield gap of winter wheat production due to P limitation is estimated to be 22% worldwide (Kvakić *et al*., 2018).

Nitrogen (N) also plays a critical role in plant biochemistry, being an integral component of many compounds, including chlorophyll, enzymes, amino acids and proteins. Generally, N is considered to be the single most important factor for determining wheat productivity and grain quality around the world (Sinclair and Rufty, 2012; Ma *et al*., 2019). However, N use efficiency (NUE) in cereals is generally poor, where it is estimated that only 30%–40% of the total of N-fertilizers applied are actually harvested in the grain (Belete *et al*., 2018). More than 100 Tg of reactive N is produced annually, of which 50% is applied to three major cereals (wheat, 18%; maize, 16%; rice, 16%) that provide the bulk of human food calories and proteins consumed either directly as grain or indirectly through livestock products (Ladha *et al*., 2016).

One approach to improve nutrient acquisition, and potentially N and P use efficiency, is to utilize beneficial plant microbes. Mycorrhizal symbioses are ubiquitous associations occurring between soil fungi and the root systems of most plant species. Benefits imparted to the

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plant due to such associations include increased mineral nutrient absorption, water-use efficiency, disease resistance, and plant-to-plant communication (Behie and Bidochka, 2014; Rillig *et al*., 2016). Nutrient transfer from the mycorrhizal partner to the plant host is crucial, especially in nutrient limiting soils (Whiteside *et al*., 2019). Consequently, studies on such symbioses wherein nutrient transfer to plants has largely focused on limiting soil nutrients, such as N and P, are critical (Averill *et al*., 2019).

The *Serendipitaceae* (Oberwinkler *et al*., 2014) is a taxonomically, physiologically and ecologically diverse family of fungi in the Basidiomycota that include endophytes and lineages that appear to have repeatedly evolved ericoid, orchid and ectomycorrhizal lifestyles (Weiss *et al*., 2016). Members of this fungal group have been shown to exhibit plant growth-promoting properties in maize (Varma *et al*., 1999), tobacco (*Nicotiana attenuate* L.) (Sherameti *et al*., 2005), switchgrass (*Panicum virgatum* L.) (Ghimire *et al*., 2009; Ray *et al*., 2015), barley (*Hordeum vulgare* L.) (Waller *et al*., 2005) and wheat (Serfling *et al*., 2007; Ray and Craven, 2016). Like the more studied arbuscular mycorrhiza (AM), *Serendipita* symbionts often provide N and P to their host plant (Sherameti *et al*., 2005; Yadav *et al*., 2010), particularly in nutrient limiting soil (Nurfadilah *et al*., 2013). Approximately 95% of the N present in the soil is in the organic form, covalently bound to carbon (C) and requiring huge amounts of energy to release a biologically available form. Intriguingly, genome analysis of *S. bescii* and *S. vermifera* suggests that *Serendipitaceae* fungi have the metabolic capacity to assimilate N from organic forms of N-containing compounds (Ray *et al*., 2019). In light of their proven beneficial impact on plant growth and nutrient acquisition in winter wheat and numerous other important crops, *Serendipitaceae* fungi should be considered as an effective tool for enhancing plant productivity and stress tolerance in nutrient poor soils. However, wider implementation of the *Serendipitaceae* fungi in agricultural contexts is limited by a scarcity of suitable strains. Our group has previously identified a new strain of *Serendipita*, *Serendipita vermifera* ssp. *bescii* (hereafter referred to as *S. bescii*), which seems to be well adapted to the specific agro-climatic conditions of the Southern Great Plains, USA (Craven and Ray, 2017; Ray *et al*., 2018b).

In this study, we used a transcriptional approach combined with root phenotyping and estimation of forage biomass to characterize the physiological responses and regulation of N and P metabolism in NF101 winter wheat when colonized by *S. bescii*. Such an approach will improve our understanding of the role of *S. bescii* in modulating host plant stress imposed by limitation of these two critical nutrients.

Results

Serendipita bescii *colonizes wheat root cells intracellularly*

Confocal microscopic images of root cells of winter wheat cultivar NF101 colonized by *S. bescii* are shown in Fig. 1. Early stages of *S. bescii* colonization are marked by sparse hyphal growth between cells. At later stages, cortical root cells became completely packed with aggregates of moniloid (bulbous) fungal hyphae. These structures resemble hyphal protrusions bearing terminally swollen parts formed by some ericoid and orchid mycorrhiza (Vohník *et al*., 2012; Ray and Craven, 2016), as well as other *Serendipitaceae* fungi (Riess *et al*., 2014).

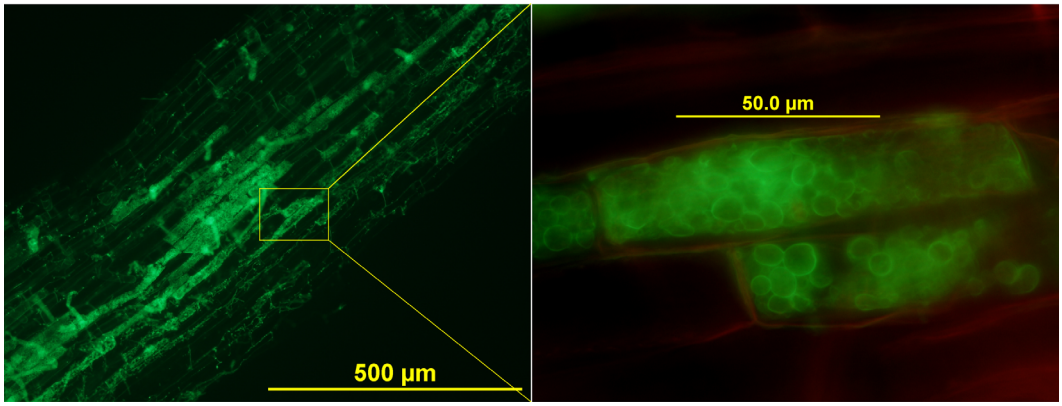

Fig 1. *Serendipita bescii* colonized root cells of winter wheat cultivar NF101 14 dpi by confocal microscopy. Colonization is typically marked by cortical root cells packed with aggregates of swollen moniloid fungal hyphae (green cells, right panel). Fungal hyphae stained with WGA-AF 488 were visualized in the green channel. Plant cell walls stained with propidium iodide were visualized in the red channel. Images from these channels were overlaid to show fungal colonization in plant cells.

Serendipita bescii *colonization improves root growth and forage biomass production*

Serendipita bescii significantly increased the number of lateral root numbers (Fig. 2) at the early stage (14 dpi) and forage biomass (Fig. 3) at later stages (74 dpi) of colonization in winter wheat under N or P-limitation, compared to uninoculated controls. The number of lateral root numbers in *S. bescii*-inoculated wheat seedlings at 14 dpi increased by 36% over uninoculated controls in either N or P limiting conditions, whereas shoot biomass at 74 dpi increased by 100% and 38% under N and P limiting conditions respectively. Importantly, these improvements were not observed when plants were treated with optimum levels of N and P. Increases in plant height due to *S. bescii* colonization at 74 dpi were numerically high, but statistically insignificant with an uninoculated plant (Fig. 3). Additionally, we did not find any significant impact on shoot nitrate or phosphate content due to *S. bescii* colonization under nutrient replete and limiting conditions.

Distribution of differentially expressed wheat genes due to S. bescii *colonization*

At 14 dpi, we identified 1516, 962 and 1159 differentially expressed (DE) wheat genes under nutrient replete, N-limitation and P-limitation conditions, respectively. Under the same conditions, at 74 dpi, we have identified 675, 149 and 165 DE genes respectively. Out of these, 534 and 447 genes at 14 dpi, 80 and 106 genes at 74 dpi were differentially expressed specifically due to *S. bescii* colonization under N- and P-limitation conditions (Supporting Information Table S1). Network analysis of all six sets of DE genes based on their expression profile is shown in Fig. 4. DE genes for all the three nutrient treatments were tightly clustered together, in a colonization stage-dependent manner (Fig. 4). Interestingly, N-limitation at the early stage is dominated by downregulated genes, whereas at the late stage, both N- and P-limitations are dominated by upregulated genes. A hierarchical clustering heatmap of DE genes due to *S. bescii*

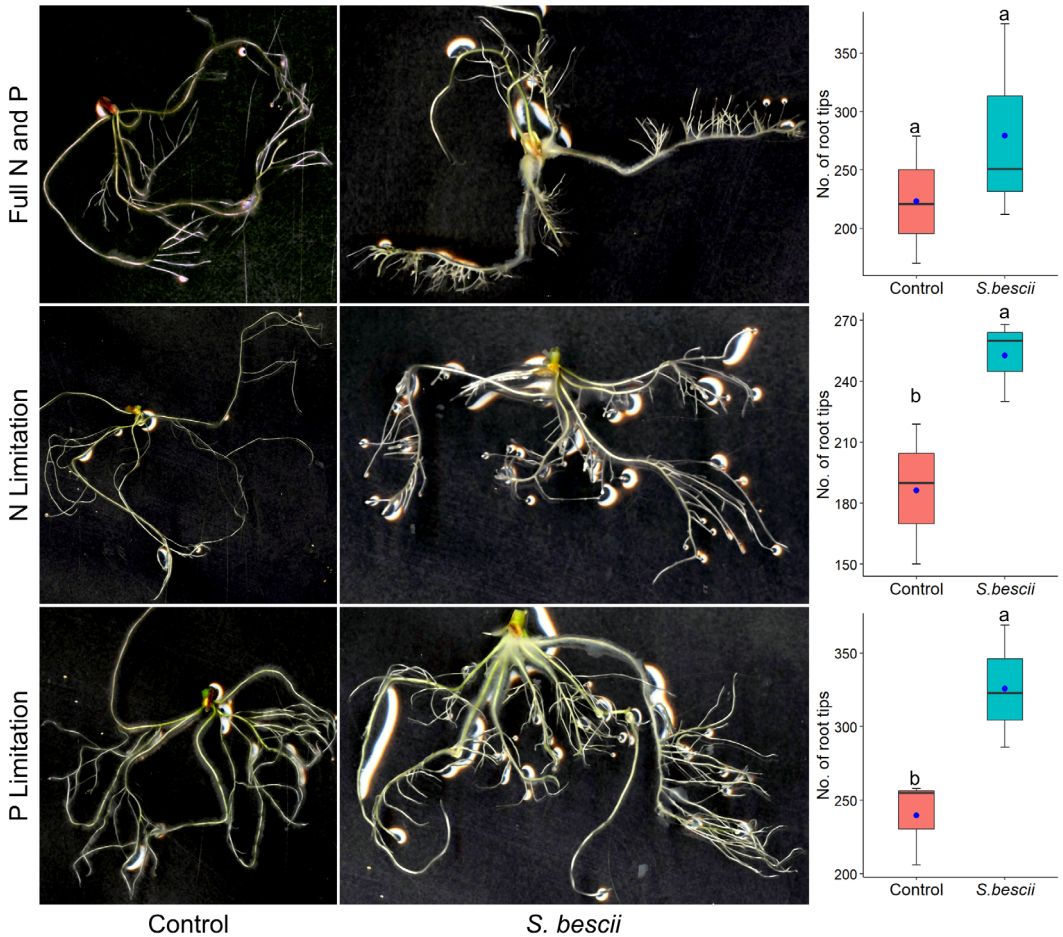

Fig 2. Effect of *S. bescii* colonization on root architecture (left) of winter wheat cultivar NF101at 14 days post inoculation *in vitro* under full N and P, N- and P-limitations. Significant increases in total number of root tips (right) were observed under N- or P-limiting conditions when colonized with *S. bescii* with respect to the uninoculated control. Blue dots denote mean values. Boxes with different letters denote significant difference between treatments at $P < 0.05$.

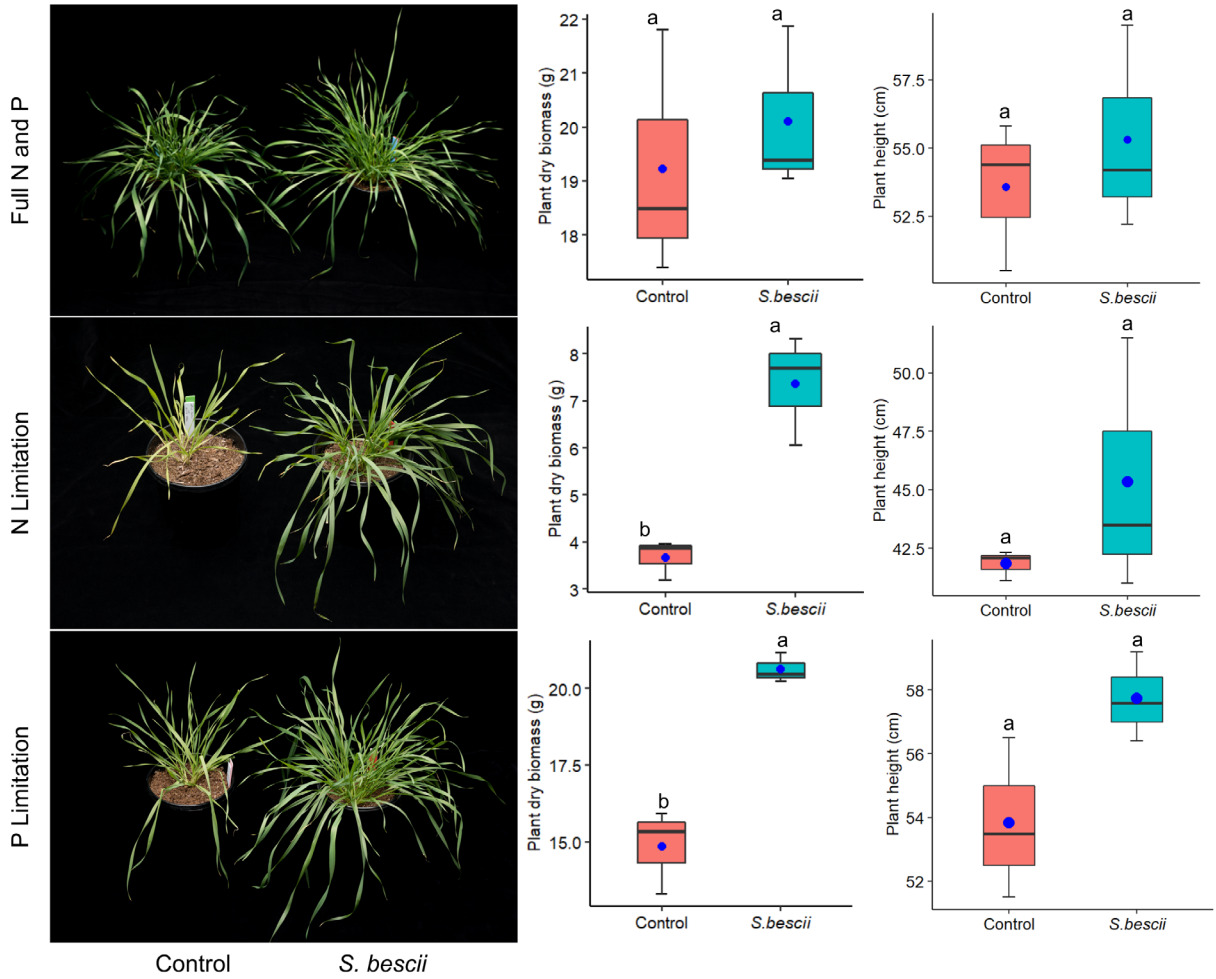

Fig 3. Effect of *S. bescii* colonization on growth (left) of winter wheat cultivar NF101at 74 days post inoculation in a greenhouse under full N and P, and N- and P-limiting conditions. A significant increase in dry forage biomass (right) production was observed under N- or P-limited condition when colonized with *S. bescii*. Increases in plant height were not significant in any of the treatments. Blue dots denote mean values. Boxes with different letters denote significant difference between treatments at $P < 0.05$.

colonization under nutrient replete, N- and P-limitation conditions, and having an absolute value of log2 fold-change difference ≥ 4 is shown in the Supporting Information Fig. S1. The DE genes showed a discrete pattern for both stages of colonization within individual nutrient treatments.

KEGG enrichment analysis of differentially expressed genes

Under nutrient replete conditions, we identified 26 and 14 KEGG pathways at 14 and 74 dpi, respectively that were enriched according to Fisher's exact test. Similarly, under the N-limitation condition, 11 and 14 pathways, and under the P-limitation condition, 27 and 20 pathways, were identified at 14 and 74 dpi, respectively (Supporitng Information Table S3). Enrichment analysis of the top 10 pathways based on *P*-value, for all three treatments and at two different stages of colonization is shown in Fig. 5.

Regulation of P and N metabolism in plants

Root architecture

Genes encoding multi-copper oxidases have been found to inhibit primary root growth under low P (Svistoonoff *et al*., 2007). Under the P-limited conditions examined here, four multi-copper oxidase genes were upregulated due to *S. bescii* at early stage of colonization (Supporting Information Table S4). Of these four genes, two were co-induced under N limitation at the same stage. Auxin has been reported to play critical roles in both root development during P starvation (Müller and Schmidt, 2004), and a nitrate-mediated signalling pathway for lateral root elongation (Sun *et al*., 2017). Several auxin-responsive genes

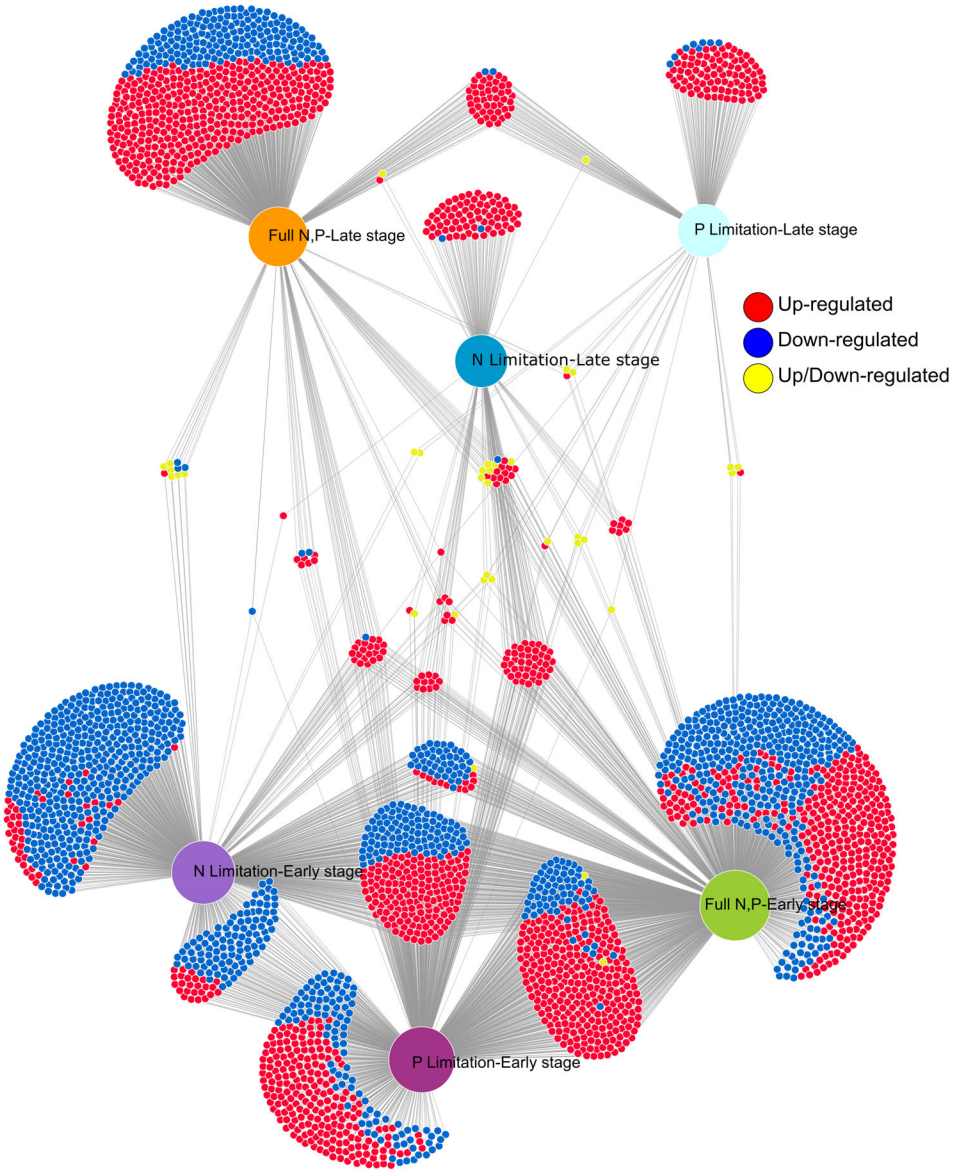

Fig 4. Network analysis of differentially expressed wheat genes due to *S. bescii* colonization at different stages under nutrient replete, and N-, and P-limitation conditions, respectively. Genes having an absolute value of log2 fold-change difference ≥ 2 were selected for the network analysis. Red and blue nodes denote upregulated and downregulated genes respectively. Yellow nodes denote up-regulation in one condition but down-regulation in the other.

were differentially expressed specifically due to *S. bescii* colonization both under nutrient replete and depleted conditions (Supporting Information Table S2). Of those, one known as AIR 12 (auxin induced in roots) (Traes_5BL_AD9944DCC) and putatively involved in lateral root morphogenesis (Gibson and Todd, 2015) was downregulated both under N or P starvation conditions during early stages of colonization. In addition, three genes, encoding auxin efflux carrier proteins that are involved in root specific auxin transport were upregulated under nutrient replete and P starvation condition at the same stage (Supporting Information Table S4). In contrast, these auxin efflux carrier proteins were downregulated in the absence of *S. bescii* suggesting a role for the fungal symbiont in regulating auxin transport.

Ethylene modulates auxin biosynthesis, transport and signalling to fine-tune root growth and development (Qin and Huang, 2018). We found three ethylene biosynthesis genes that were specifically downregulated during the early stage of colonization under N and P starvation conditions (Supporting Information Table S4). One root hair-specific gene (Traes_3DL_2F30F7D2D) putatively involved in root hair elongation was specifically downregulated at the early stage of colonization due to either N or P starvation.

Phosphate (Pi) transport and utilization

Hydrolases such as phosphatases and ribonucleases are involved in releasing Pi from organic sources and are

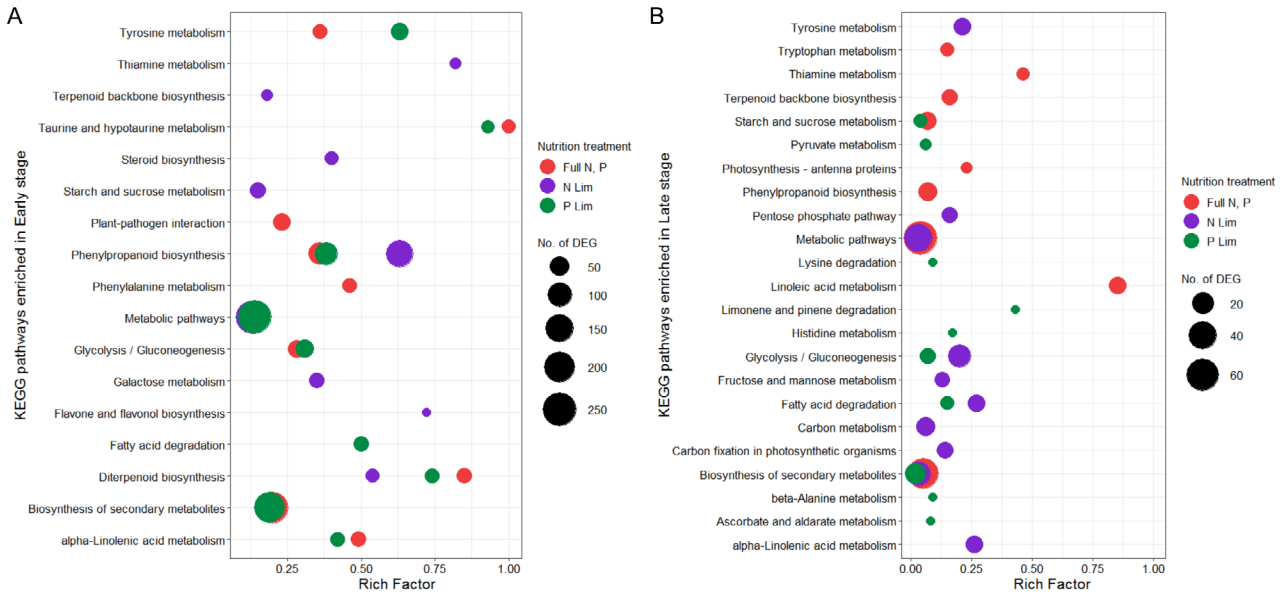

Fig 5. Scatter plot of the most enriched KEGG pathways, for all three nutrient treatments at (A) early and (B) late stages of colonization. Rich factor is the ratio of the number of differentially expressed genes to the total gene number in a certain pathway. The rich factor value is directly proportional to the degree of enrichment. The colour and size of the dots represent nutrition treatment, and the number of differentially expressed genes mapped to the indicated pathways, respectively. Top 10 enriched pathways based on FDR-corrected *P*-value ($P < 0.05$) are shown.

greatly induced by P deficiency in model plant *Arabidopsis* and rice (Plaxton and Tran, 2011). We found one (Traes_5BS_F389AB55C) ribonuclease H-like superfamily protein putatively involved in triacylglycerol degradation, and one purple acid phosphatase gene (Traes_2BL_859881F87) that were specifically downregulated at the early stage of colonization under P-starvation conditions (Supporting Information Table S4).

N uptake and remobilization

Plants take up nitrate as the preferred source of N when it is available in abundance (Crawford and Glass, 1998). When *S. bescii* was absent, three nitrate transporters were induced upon N-limitation. However, when the fungus was present, all of them were repressed in under nutrient replete condition, whereas none of them was differentially expressed under N-depleted condition (Supporting Information Table S2). Conversely, we identified two ammonium transporters in the plant (Traes_6AL_CCF2A8825, Traes_6DL_3286E5385) that were specifically downregulated under N-limiting conditions, again when colonized by *S. bescii* (Supporting Information Table S4).

After entering the plant cell, ammonium is eventually transformed into the key N donor glutamine, via the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway. We identified a SNF1-related protein kinase gene (Traes_3DL_6103E4919), putatively involved in nitrate assimilation, and an amidase gene (Traes_2DS_6B79F7E51) that acts on glutamine to free up cellular N to be up- and downregulated respectively under N-starvation, only when *S. bescii* was present (Supporting Information Table S4).

Autophagy and the ubiquitin-proteasome mechanism are the two major protein degradation pathways in plants, potentially liberating N as a consequence. In this study, we found three each of aspartyl proteases and cysteine proteinases genes putatively involved in the autophagy pathway that were specifically downregulated due to *S. bescii* colonization under N-starvation conditions (Supporting Information Table S4). After protein degradation, released amino acids are typically loaded into phloem vessels by amino acid permeases (AAP) and oligopeptide transporters (OPT). We found two AAPs and three OPTs to be downregulated due to *S. bescii* at the early stage of colonization under N-starvation (Supporting Information Table S4).

The MtN21 protein-encoding genes are reported to act as bidirectional transporters of amino acids such as glutamine and are able to export amino acids out of cells when necessary (Ladwig *et al*., 2012). We found, two MtN21 genes (Traes_3AS_3B1466678, Traes_3B_848719122) to be down- and one upregulated (Traes_5BL_CBB9D7E7F) under the N limitation condition at 14 and 74 dpi respectively (Supporting Information Table S4).

Biosynthesis of phytohormones and secondary metabolites

Strigolactones are carotenoid-derived plant hormones and are recognized as important local and systemic

signals involved in the regulation of shoot architecture and acclimatization of plants to P-limiting conditions (Czarnecki *et al*., 2013; Kapulnik and Koltai, 2016). Additionally, abscisic acid (ABA) and gibberellic acid (GA) are involved in P starvation signalling in plants (Baek *et al*., 2017). ABA has also been implicated in the nitrate-mediated signalling pathway for lateral root elongation (Harris and Ondzighi-Assoume, 2017). In our study, two putative strigolactone esterase D14 genes (Traes_4BL_D05623E45, Traes_4BL_D62825306) involved in the strigolactone signalling pathway, and one GA 2-oxidase 6 gene (Traes_1DS_125A395C1) was upregulated under P starvation condition at the later stage of colonization (Supporting Information Table S4).

P starvation typically induces the regulation of secondary metabolites such as glucosinolates, flavonoids and phenylpropanoid compounds such as anthocyanins (Steyn *et al*., 2002; Jiang *et al*., 2007). A flavonoid biosynthesis gene (Traes_4AS_E7B757DEA) and an anthocyanin biosynthesis gene (Traes_4BL_8AA727112) were found be downregulated specifically due to *S. bescii* at 14 dpi under both P- and N-limiting conditions. Additionally, three sulfotransferase genes that are putatively involved in biosynthesis of glucosinolates were upregulated when *S. bescii* was present at 74 dpi, under P-limitation conditions (Supporting Information Table S4).

Membrane lipid remodelling

Membrane lipid remodelling during P starvation consists of degradation of phospholipids, to release Pi and the synthesize glycolipids to substitute for phospholipids (Pant *et al*., 2015). We found, genes involved in degradation of phospholipids (Traes_3B_5B8189B41, Traes_7BL_11E10C066), glycerolipid (Traes_3B_8E820A790, Traes_3B_C5168A652) and fatty acid biosynthesis (Traes_4AS_080C79870, Traes_4DL_74F91F3BD) that were upregulated upon P-starvation when the fungal symbiont was present (Supporting Information Table S4).

Discussion

Serendipita bescii *colonization promotes host plant growth*

Serendipitaceae fungi often provide N and P to the host plant, particularly in nutrient-limited conditions and in orchids and ericaceous plants (Nurfadilah *et al*., 2013; Wu *et al*., 2019). In this study, we report the plant growth promoting potential of *S. bescii*, an endophyte we isolated from switchgrass roots and inoculated onto the roots of winter wheat under N- or P-limiting conditions. When *S. bescii* was present, we found a significant increase in both the number of lateral root numbers in young wheat seedlings and forage biomass in more mature plants, specifically under nutrient-depleted conditions in *S. bescii* colonized plants. This finding is consistent with the AM symbiosis, wherein the fungus promotes plant growth under suboptimal N and P, but incongruent with *S. indica* (a close relative of *S. bescii*), whose influence on plant growth promotion seems to be independent of N and P levels (Achatz *et al*., 2010). In this study, we did not record any *S. bescii*-induced plant growth promotion under optimum N and P conditions. Further, Barazani *et al*. (2005) and Achatz *et al*. (2010) showed that *S. indica* did not significantly increase shoot N and P content in tobacco and barley. We also did not find any significant impact on shoot nitrate or phosphate acquisition due to *S. bescii* colonization under nutrient replete and limiting conditions. This could be primarily due to the fact that, shoot nutrient acquisition and plant growth promotion are not necessarily positively correlated phenotypic traits.

Regulation of P starvation response in winter wheat by S. bescii

Plants respond to P-limitation with a variety of morphological, physiological, biochemical and molecular changes collectively called the phosphate starvation response (PSR), which may improve P acquisition and/or mobilization leading to improved P use efficiency. In this study, we propose that *S. bescii* modulates at least some of the PSR response in winter wheat, such as a reduction in primary root elongation and a corresponding increase in the number of lateral roots. This is a critical part of the PSR, trait employed by plants to increase their effective root surface area in search of available Pi (López-Bucio *et al*., 2002; Chevalier *et al*., 2003). *Serendipita bescii* colonization under P starvation upregulated four multicopper oxidase genes (Supporting Information Table S4) known to be involved in primary root inhibition, as well as increased lateral root numbers suggesting their role in modulating the PSR.

Genes encoding plant hormones not only play critical roles in plant growth and development but also in plant-microbe symbioses. P-starvation typically impact plant hormone levels, with auxin, ethylene and strigolactones categorized as low P-induced hormones that positively regulate the PSR, and cytokinins and gibberellins are known as low P-repressed hormones that negatively impact the PSR (Scheible and Rojas-Triana, 2018). Partially in support of this notion, we found that P starvation in plants colonized by *S. bescii* induced the upregulation of genes involved in root-specific auxin transport, and, the strigolactone signalling pathway, both positive PSR regulators, and GA 2-oxidase 6 gene responsible for inactivation of bioactive GA. Several reports suggest that

such inactivation of bioactive GA leads to increasing tillering in monocots such as rice and switchgrass (Lo *et al*., 2008; Wuddineh *et al*., 2015). While this is not the subject of the current study, but we have consistently seen increased tillering in switchgrass and winter wheat when colonized with *S. bescii* both in greenhouse and field conditions.

When Pi is unavailable, plants synthesize certain types of ribonucleases (RNAses) and purple acid phosphatases (PAPs) that can access and mobilize phosphate from RNA and other organic compounds during P starvation. We found that *S. bescii* colonization downregulated RNAses and PAPs under P-limiting conditions suggesting its role in host P remobilization.

Finally, phospholipids are fundamental components of plant membranes and, during phosphate starvation, phospholipids are degraded, and conversely, a non-phosphorus galactolipids accumulates in the root plasma membrane of plants (Nakamura *et al*., 2005) leading to the release of Pi needed by the plant cells. In our study, plants with *S. bescii* and grown under limiting P, upregulated genes involved in glycerolipid biosynthesis and genes responsible for degradation of phospholipids. This suggests that *S. bescii* may play a critical role in host lipid metabolism, at least under P-limiting conditions.

Serendipita bescii *colonization modulates N metabolism in winter wheat*

N deprivation has multiple consequences for plant fitness, including a decreased rate of photosynthesis, coupled to an increased translocation of photosynthate to roots, resulting in a decreased shoot:root ratio. Macroscopically, we observed yellowing of leaves and a severe reduction in forage biomass of uninoculated wheat plants grown under N- and P-limitation. Significantly, these phenotypes were largely alleviated when the fungal symbiont was present (Fig. 3).

In terms of gene expression, N starvation conditions in plants inoculated with *S. bescii*, exhibited a downregulation of ammonium transporters and genes involved in nitrate assimilation in plant cells. To maintain essential cellular functions for surviving in an N-limiting environment, plants release N from leaves, grain, and other source tissues to meet its requirement (Kant *et al*., 2010). Amino acids released from protein degradation are eventually loaded into the phloem for remobilization from source to sink tissues. We found that under N-limited conditions, *S. bescii* colonization seemingly controlled the degradation of storage proteins by downregulating several genes belonging to aspartyl proteases and cysteine proteinases known involved in autophagy. Further, colonized plants repressed several amino acid and oligopeptide transporters involved in amino acid translocation, restricting N-remobilization. These findings along with improved biomass data (Fig. 3) support a role for *S. bescii* in dealing with N-limitation and improving plant growth through a distinct, as of yet unknown mechanism.

To conclude, we show that *S. bescii* colonization increases the number of lateral roots and improved forage biomass in winter wheat under a nutrient-limited regime. To alleviative host responses related to nutrient limitation, *S. bescii* activates plant genes involved in the adaptive P and/or N starvation response to help the host plant survive under nutrient limiting conditions. Based on this transcriptome regulation and the observed physiological responses to N or P limitation, we suggest that *S. bescii* has the potential to alleviate stress imposed by limitation of these two critical nutrients in winter wheat.

Experimental procedure

Fungal material and culture conditions

Serendipita bescii strain NFPB0129 was isolated from a switchgrass plot located in Oklahoma, USA (Ray *et al*., 2018b) and used in this study. The strain was routinely maintained in Modified Melin Norkan's (MMN) media (Marx, 1969), with 1% glucose as a C source at pH 6.0 in 24°C.

Plant material

Seeds of hard red winter wheat cultivar NF101 were surface sterilized following the protocol modified after Xi *et al*. (2009). Briefly, seeds were washed with 50% Clorox® (8.25% sodium hypochlorite, Clorox, Oakland, CA, USA) containing 0.1% TWEEN®20 (Amresco, Solon, OH, USA) for 30 min. After three washes with sterile water, seeds were soaked in sterile water and kept at 4°C overnight. Next day, seeds were treated one additional time with 50% Clorox® for 30 min, washed with sterile water for three times and air dried on sterile filter paper.

Colonization of wheat seedlings in vitro

In vitro colonization was performed using Magenta™ GA-7 plant culture boxes (7.5 × 7.5 × 10 cm) with lids (Magenta LLC, Lockport, IL, USA). The boxes were filled with 100 ml of M medium (Schultze, 2013) pH 5.5, containing 0.3% phytagel® and amended with 1% sucrose. N and P starvation were imposed by preparing M Media without any nitrate or phosphate respectively. M media supplemented with nitrate and phosphate were prepared as controls for direct comparison. Surface sterilized wheat seeds were plated onto these media.

Inoculum of *S. bescii* was prepared by following the protocol described in the study by Ray *et al*. (2018a). Briefly, starter culture for inoculation was prepared by growing *S. bescii* in 5 ml of standard M media for 2 weeks in 50 ml Falcon tube. After 2 weeks, mycelia was harvested by brief low speed centrifugation (5000*g*; 2 min), washed with sterile water for three times to get rid of residual media. Subsequently, the inoculum was prepared by grinding 50 mg of fresh, washed vegetative mycelia in 500 μl of sterile water. *In vitro* colonization was achieved by injecting 20 μl of aqueous mycelial suspension directly into the media with a sterile pipette (Ray *et al*., 2015). Seedlings were maintained in a TCR-180 Conviron® growth chamber at 24°C, 50% RH and 16 h photoperiod for 2 weeks (Controlled Environments Ltd, Winnipeg, MB, Canada). After 2 weeks, a subset of *in vitro* colonized and the corresponding uninoculated control plants were transplanted into one-gallon trade pots (2.68 l, Stuewe and Sons, Inc. Tangent, OR, USA) filled with Metro-Mix®360 (Sun Gro® Horticulture, Agawam, MA, USA) and transferred to the greenhouse. The Metro-Mix®360 was pre washed with clear water to get rid of residual N or P. The plants were maintained in the greenhouse for 60 days (24°C, 50% RH, 16 h photoperiod), i.e. 74 days post inoculation (dpi) and watered with either optimum, or N- or P-deficient ½ strength Hoagland's solution (Caisson Labs, Smithfield, UT, USA) respectively. The entire experiment was conducted with three biological replicates for each treatment

Visualization of S. bescii *colonization by confocal microscopy*

Colonization of wheat roots by *S. bescii* was confirmed by fluorescence staining and confocal microscopy following the protocol described in the study by Ray *et al*. (2018a). Briefly, colonized roots were fixed in 50% ethanol, cleared with 20% KOH, and stained with WGA-AF 488® (Life Technologies, Carlsbad, CA, USA), followed by counter staining with propidium iodide (PI: Biotium, Hayward, CA, USA) to visualize fungal mycelia and plant cell wall respectively. Stained root samples were visualized with a Leica TCS-SP8 point scanning confocal microscope (Leica Microsystems, Wetzlar, Germany) (Fig. 1).

Estimation of root and forage biomass production

Roots were harvested at 14 dpi from *in vitro* culture boxes and the total number of root tips were counted using the WinRHIZO™ Pro (Regent Instruments Canada) root image analysis software following the manufacturer's instructions. Shoot biomass was harvested from greenhouse grown plants at 74 dpi. Within each nutrient treatment, the data were analysed by one-way ANOVA using CoStat statistical software 6.4 (Cohort Berkeley, CA, USA). Treatment means were compared using LSD value at $P \leq 0.05$. The data were plotted graphically using the package ggplot2 (Wickham, 2016) in R studio® (R Studio, Boston, MA).

RNA sequencing and data analysis

At harvest (14 dpi, early stage; 74 dpi, late stage), total RNA was isolated from root tissues using an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). RNA-seq libraries were prepared using TruSeq Stranded mRNA sample preparation kit (Illumina, San Diego, CA). Individual libraries were uniquely indexed using TruSeq single indexes (Illumina), and pooled in equimolar ratio. The pooled libraries were sequenced on a NextSeq 500 Sequencing system (Illumina).

The reads for each sample were quality trimmed to remove any low quality bases and primer/adapter sequences. Reads less than 30 bases long after trimming were discarded, along with their mate pair. The trimmed reads were then mapped to version 2.2 of the *T. aestivum* genome (Phytozome v12.1) using HISAT2 (Kim *et al*., 2015). The identified transcripts were compared with the reference genome's set of transcripts using Stringtie (Pertea *et al*., 2015). Differential expression testing was performed using DESeq2 (Love *et al*., 2014). To identify genes whose expression was regulated in response to *S. bescii* colonization in the nutrient replete condition and either of the N- or P-depleted conditions, we performed pairwise comparisons and only considered genes having log2 fold change ≥2 and adjusted *P*-value <0.05 as biologically significant (Supporting Information Table S2). RNA-seq data have been submitted to the DDBJ/EMBL/GenBank databases under accession number PRJNA573046.

Network analysis and hierarchical clustering of differentially expressed genes

Visualization of biologically significant wheat genes based on their expression data across all the treatments and two time points were done in a force-directed graph to infer the relationship network using the web-based visualization tool Divenn (Sun *et al*., 2019). Up or downregulated genes were represented by red or blue nodes respectively. The yellow node denotes genes upregulated in one treatment but downregulated in the other (Fig. 4).

Hierarchical clustering of differentially expressed genes having an absolute value of log2 fold-change difference ≥ 4 was done to depict global changes of differentially expressed genes across all treatments and time points. The heat map representation of hierarchical

clustering was generated using the function heatmap.2 in the package gplots (Warnes *et al*., 2016) in R studio® (R Studio) (Supporting Information Fig. S1).

KEGG pathway enrichment analysis of wheat genes

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of wheat genes was done using KOBAS 3.0 (Xie *et al*., 2011). Enrichment analysis was done based on Fisher's exact test with FDR-corrected *P*-value <0.05. The rich factor is calculated as the ratio of the numbers of DEGs enriched in this pathway, to the total number of genes annotated in the same pathway. Top 10 pathways based on FDR-corrected *P*-values, for all three treatments at two different stages of colonization were plotted graphically using the package ggplot2 (Wickham, 2016) in R studio® (R Studio) (Fig. 5).

qRT-PCR validation of candidate genes

Quantitative RT-PCR was performed with three replicates from independent biological experiments to verify differential transcription of the candidate genes following the protocol described in the study by Yi *et al*. (2009), using QuantiTect®RT-kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction (Supporting Information Fig. S2). Real-time PCR was run on the Applied Biosystems QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Normalization and comparison of mean CT (threshold cycle) values were calculated following the method by Livak and Schmittgen (2001). To compare relative abundance of transcripts of target genes, the mean CT of triplicate reactions was normalized by wheat actin 2 (wACT2), which is one of the most stably expressed genes (Tenea *et al*., 2011).

Estimation of relative abundance of S. bescii *in planta*

The relative abundance of *S. bescii* in the different nutrient treatments and across different time points was estimated by qPCR. The wheat ACT2 (wACT2) (Tenea *et al*., 2011) and *S. bescii* ACT1 (sACT1) genes were used as indicators for calculation of relative abundance. Based on haploid genome size of each organism (wheat-34Gb, *S. bescii*-37 Mb) and their ploidy as well as karyotype and C-value (Dolezel, 2003), we calculated that 23 ng of wheat gDNA and 0.3 ng of *S. bescii* gDNA will contain similar numbers of ACT gene templates (7953 copies and 7929 copies respectively). Based on this calculation, a dilution series of *S. bescii* gDNA (0.24, 0.18, 0.12, 0.06 ng) was prepared by mixing each with 23 ng of wheat gDNA, and the CT values were measured to generate a standard curve (*S. bescii*/wheat template ratio (%) $= 98.03922 \times 2^{-\Delta Ct}$, $R^2 = 0.09901$). Finally, the relative abundance of *S.bescii* in wheat root was quantified by using this standard curve from the ΔCt value of the root DNA (Supporting Information Fig. S3).

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References

Achatz, B., von Rüden, S., Andrade, D., Neumann, E., Pons-Kühnemann, J., Kogel, K.-H., *et al*. (2010) Root colonization by Piriformospora indica enhances grain yield in barley under diverse nutrient regimes by accelerating plant development. *Plant Soil* **333**: 59–70.

Averill, C., Bhatnagar, J.M., Dietze, M.C., Pearse, W.D., and Kivlin, S.N. (2019) Global imprint of mycorrhizal fungi on whole-plant nutrient economics. *Proc Natl Acad Sci* **116**: 23163–23168.

Baek, D., Chun, H.J., Yun, D.-J., and Kim, M.C. (2017) Cross-talk between phosphate starvation and other environmental stress signaling pathways in plants. *Mol Cells* **40**: 697.

Barazani, O., Benderoth, M., Groten, K., Kuhlemeier, C., and Baldwin, I.T. (2005) Piriformospora indica and Sebacina vermifera increase growth performance at the expense of herbivore resistance in *Nicotiana attenuata*. *Oecologia* **146**: 234–243.

Behie, S.W., and Bidochka, M.J. (2014) Nutrient transfer in plant–fungal symbioses. *Trends Plant Sci* **19**: 734–740.

Belete, F., Dechassa, N., Molla, A., and Tana, T. (2018) Effect of nitrogen fertilizer rates on grain yield and nitrogen uptake and use efficiency of bread wheat (*Triticum aestivum* L.) varieties on the Vertisols of central highlands of Ethiopia. *Agric Food Security* **7**: 78.

Chevalier, F., Pata, M., Nacry, P., Doumas, P., and Rossignol, M. (2003) Effects of phosphate availability on the root system architecture: large-scale analysis of the natural variation between Arabidopsis accessions. *Plant Cell Environ* **26**: 1839–1850.

Craven, K., and Ray, P. (2017) Symbiont for enhancement of plant performance. US Patent App. 15/626,926. https://patents.google.com/patent/WO2017210651A1/en

Crawford, N.M., and Glass, A.D. (1998) Molecular and physiological aspects of nitrate uptake in plants. *Trends Plant Sci* **3**: 389–395.

Czarnecki, O., Yang, J., Weston, D., Tuskan, G., and Chen, J.-G. (2013) A dual role of strigolactones in phosphate acquisition and utilization in plants. *Int J Mol Sci* **14**: 7681–7701.

Dolezel, J. (2003) Nuclear DNA content and genome size of trout and human. *Cytometry A* **51**: 127–128.

Ghimire, S.R., Charlton, N.D., and Craven, K.D. (2009) The mycorrhizal fungus, Sebacina vermifera, enhances seed germination and biomass production in switchgrass (*Panicum virgatum* L). *Bioenergy Res* **2**: 51–58.

Gibson, S.W., and Todd, C.D. (2015) Arabidopsis AIR12 influences root development. *Physiol Mol Biol Plants* **21**: 479–489.

Harris, J.M., and Ondzighi-Assoume, C.A. (2017) Environmental nitrate signals through abscisic acid in the root tip. *Plant Signal Behav* **12**: e1273303.

Jiang, C., Gao, X., Liao, L., Harberd, N.P., and Fu, X. (2007) Phosphate starvation root architecture and anthocyanin accumulation responses are modulated by the gibberellin-DELLA signaling pathway in Arabidopsis. *Plant Physiol* **145**: 1460–1470.

Kant, S., Bi, Y.-M., and Rothstein, S.J. (2010) Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *J Exp Bot* **62**: 1499–1509.

Kapulnik, Y., and Koltai, H. (2016) Fine-tuning by strigolactones of root response to low phosphate. *J Integr Plant Biol* **58**: 203–212.

Kim, D., Langmead, B., and Salzberg, S.L. (2015) HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* **12**: 357–360.

Kvakić, M., Pellerin, S., Ciais, P., Achat, D.L., Augusto, L., Denoroy, P., *et al*. (2018) Quantifying the limitation to world cereal production due to soil phosphorus status. *Global Biogeochem Cycles* **32**: 143–157.

Ladha, J., Tirol-Padre, A., Reddy, C., Cassman, K., Verma, S., Powlson, D., *et al*. (2016) Global nitrogen budgets in cereals: a 50-year assessment for maize, rice, and wheat production systems. *Sci Rep* **6**: 19355.

Ladwig, F., Stahl, M., Ludewig, U., Hirner, A.A., Hammes, U. Z., Stadler, R., *et al*. (2012) Siliques are Red1 from Arabidopsis acts as a bidirectional amino acid transporter that is crucial for the amino acid homeostasis of siliques. *Plant Physiol* **158**: 1643–1655.

Livak, K.J., and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods* **25**: 402–408.

Lo, S.-F., Yang, S.-Y., Chen, K.-T., Hsing, Y.-I., Zeevaart, J. A., Chen, L.-J., and Yu, S.-M. (2008) A novel class of gibberellin 2-oxidases control semidwarfism, tillering, and root development in rice. *Plant Cell* **20**: 2603–2618.

López-Bucio, J., Hernández-Abreu, E., Sánchez-Calderón, L., Nieto-Jacobo, M.F., Simpson, J., and Herrera-Estrella, L. (2002) Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. *Plant Physiol* **129**: 244–256.

Love, M.I., Huber, W., and Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**: 550.

Ma, G., Liu, W., Li, S., Zhang, P., Wang, C., Lu, H., *et al*. (2019) Determining the optimal N input to improve grain yield and quality in winter wheat with reduced apparent N loss in the North China plain. *Front Plant Sci* **10**: 181.

Marx, D.H. (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* **59**: 153–163.

Müller, M., and Schmidt, W. (2004) Environmentally induced plasticity of root hair development in Arabidopsis. *Plant Physiol* **134**: 409–419.

Nakamura, Y., Awai, K., Masuda, T., Yoshioka, Y., Takamiya, K.-i., and Ohta, H. (2005) A novel phosphatidylcholine-hydrolyzing phospholipase C induced by phosphate starvation in Arabidopsis. *J Biol Chem* **280**: 7469–7476.

Nurfadilah, S., Swarts, N.D., Dixon, K.W., Lambers, H., and Merritt, D.J. (2013) Variation in nutrient-acquisition patterns by mycorrhizal fungi of rare and common orchids explains diversification in a global biodiversity hotspot. *Ann Bot* **111**: 1233–1241.

Oberwinkler, F., Riess, K., Bauer, R., and Garnica, S. (2014) Morphology and molecules: the Sebacinales, a case study. *Mycol Prog* **13**: 445–470.

Pant, B.D., Burgos, A., Pant, P., Cuadros-Inostroza, A., Willmitzer, L., and Scheible, W.-R. (2015) The transcription factor PHR1 regulates lipid remodeling and triacylglycerol accumulation in *Arabidopsis thaliana* during phosphorus starvation. *J Exp Bot* **66**: 1907–1918.

Patrignani, A., Lollato, R.P., Ochsner, T.E., Godsey, C.B., and Edwards, J. (2014) Yield gap and production gap of rainfed winter wheat in the southern Great Plains. *Agron J* **106**: 1329–1339.

Pertea, M., Pertea, G.M., Antonescu, C.M., Chang, T.-C., Mendell, J.T., and Salzberg, S.L. (2015) StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol* **33**: 290–295.

Pierzynski, G.M., McDowell, R.W., and Thomas Sims, J. (2005). Chemistry, cycling, and potential movement of inorganic phosphorus in soils. *Phosphorus: Agriculture and the environment*, **46**: 51–86.

Plaxton, W.C., and Tran, H.T. (2011) Metabolic adaptations of phosphate-starved plants. *Plant Physiol* **156**: 1006–1015.

Qin, H., and Huang, R. (2018) Auxin controlled by ethylene steers root development. *Int J Mol Sci* **19**: 3656.

Ray, P., and Craven, K.D. (2016) Sebacina vermifera: a unique root symbiont with vast agronomic potential. *World J Microbiol Biotechnol* **32**: 1–10.

Ray, P., Guo, Y., Kolape, J., and Craven, K.D. (2018a) Non-targeted colonization by the Endomycorrhizal fungus, Serendipita vermifera, in three weeds typically co-occurring with Switchgrass. *Front Plant Sci* **8**: 2236.

Ray, P., Ishiga, T., Decker, S.R., Turner, G.B., and Craven, K.D. (2015) A novel delivery system for the root symbiotic fungus, Sebacina vermifera, and consequent biomass enhancement of low lignin COMT Switchgrass lines. *Bioenergy Res* **8**: 922–933.

Ray, P., Chi, M.-H., Guo, Y., Chen, C., Adam, C., Kuo, A., *et al*. (2018b) Genome sequence of the plant growth-promoting fungus Serendipita vermifera subsp. bescii: the first native strain from North America. In *Phytobiomes*, Vol. **2**, pp. 62–63. https://apsjournals.apsnet.org/doi/pdf/10.1094/PBIOMES-04-17-0017-A.

Ray, P., Abraham, P.E., Guo, Y., Giannone, R.J., Engle, N.L., Yang, Z.K., *et al*. (2019) Scavenging organic nitrogen and remodeling lipid metabolism are key survival strategies adopted by the endophytic fungi, Serendipita vermifera and

Serendipita bescii to alleviate nitrogen and phosphorous starvation in vitro. *Environ Microbiol Rep* **0**, 548–557.

Riess, K., Oberwinkler, F., Bauer, R., and Garnica, S. (2014) Communities of endophytic Sebacinales associated with roots of herbaceous plants in agricultural and grassland ecosystems are dominated by Serendipita herbamans sp. nov. *PLoS One* **9**: e94676.

Rillig, M.C., Sosa-Hernández, M.A., Roy, J., Aguilar-Trigueros, C.A., Vályi, K., and Lehmann, A. (2016) Towards an integrated mycorrhizal technology: harnessing mycorrhiza for sustainable intensification in agriculture. *Front Plant Sci* **7**: 1625.

Scheible, W.R., and Rojas-Triana, M. (2018) Sensing, signalling, and control of phosphate starvation in plants: molecular players and applications. *Annu Plant Rev Online*, **48**: 23–63. https://doi.org/10.1002/9781118958841.ch2.

Schultze, M. (2013) Protocols for growing plant symbioses; mycorrhiza. *Methods Mol Biol*, **953**: 47–59. https://doi.org/10.1007/978-1-62703-152-3_3.

Serfling, A., Wirsel, S.G., Lind, V., and Deising, H.B. (2007) Performance of the biocontrol fungus Piriformospora indica on wheat under greenhouse and field conditions. *Phytopathology* **97**: 523–531.

Sherameti, I., Shahollari, B., Venus, Y., Altschmied, L., Varma, A., and Oelmüller, R. (2005) The endophytic fungus Piriformospora indica stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and Arabidopsis roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. *J Biol Chem* **280**: 26241–26247.

Sinclair, T.R., and Rufty, T.W. (2012) Nitrogen and water resources commonly limit crop yield increases, not necessarily plant genetics. *Glob Food Sec* **1**: 94–98.

Steyn, W., Wand, S., Holcroft, D., and Jacobs, G. (2002) Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. *New Phytol* **155**: 349–361.

Sun, C.-H., Yu, J.-Q., and Hu, D.-G. (2017) Nitrate: a crucial signal during lateral roots development. *Front Plant Sci* **8**: 485.

Sun, L., Dong, S., Ge, Y., Fonseca, J.P., Robinson, Z.T., Mysore, K.S., and Mehta, P. (2019) DiVenn: an interactive and integrated web-based visualization tool for comparing gene lists. *Front Genet* **10**: 421.

Svistoonoff, S., Creff, A., Reymond, M., Sigoillot-Claude, C., Ricaud, L., Blanchet, A., *et al*. (2007) Root tip contact with low-phosphate media reprograms plant root architecture. *Nat Genet* **39**: 792–796.

Tenea, G.N., Bota, A.P., Raposo, F.C., and Maquet, A. (2011) Reference genes for gene expression studies in wheat flag leaves grown under different farming conditions. *BMC Res Notes* **4**: 373.

Varma, A., Verma, S., Sahay, N., Bütehorn, B., and Franken, P. (1999) Piriformospora indica, a cultivable plant-growth-promoting root endophyte. *Appl Environ Microbiol* **65**: 2741–2744.

Vohník, M., Sadowsky, J.J., Kohout, P., Lhotáková, Z., Nestby, R., and Kolařík, M. (2012) Novel root-fungus symbiosis in Ericaceae: sheathed ericoid mycorrhiza formed by a hitherto undescribed basidiomycete with affinities to Trechisporales. *PLoS one* **7**: e39524.

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., *et al*. (2005) The endophytic fungus Piriformospora indica reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc Natl Acad Sci* **102**: 13386–13391.

Warnes, G.R., Bolker, B., Bonebakker, L., Gentleman, R., Liaw, W.H.A., Lumley, T. et al. (2016) Gplots: various R programming tools for plotting data. R package version 3.0. 1. The Comprehensive R Archive Network.

Weiss, M., Waller, F., Zuccaro, A., and Selosse, M.A. (2016) Sebacinales–one thousand and one interactions with land plants. *New Phytol* **211**: 20–40.

Whiteside, M.D., Werner, G.D., Caldas, V.E., van't Padje, A., Dupin, S.E., Elbers, B., *et al*. (2019) Mycorrhizal fungi respond to resource inequality by moving phosphorus from rich to poor patches across networks. *Curr Biol* **29**: 2043–2050.

Wickham, H. (2016) *ggplot2: Elegant Graphics for Data Analysis*, New York: Springer.

Wu, C., Li, B., Wei, Q., Pan, R., and Zhang, W. (2019) Endophytic fungus Serendipita indica increased nutrition absorption and biomass accumulation in *Cunninghamia lanceolata* seedlings under low phosphate. *Acta Ecol Sin* **39**: 21–29.

Wuddineh, W.A., Mazarei, M., Zhang, J., Poovaiah, C.R., Mann, D.G., Ziebell, A., *et al*. (2015) Identification and overexpression of gibberellin 2-oxidase (GA 2ox) in switchgrass (P anicum virgatum L.) for improved plant architecture and reduced biomass recalcitrance. *Plant Biotechnol J* **13**: 636–647.

Xi, Y., Fu, C., Ge, Y., Nandakumar, R., Hisano, H., Bouton, J., and Wang, Z.-Y. (2009) Agrobacterium-mediated transformation of switchgrass and inheritance of the transgenes. *Bioenergy Res* **2**: 275–283.

Xie, C., Mao, X., Huang, J., Ding, Y., Wu, J., Dong, S., *et al*. (2011) KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res* **39**: W316–W322.

Yadav, V., Kumar, M., Deep, D.K., Kumar, H., Sharma, R., Tripathi, T., *et al*. (2010) A phosphate transporter from the root endophytic fungus Piriformospora indica plays a role in the phosphate transport to the host plant. *J Biol Chem* **M110**: 111021.

Yi, M., Chi, M.-H., Khang, C.H., Park, S.-Y., Kang, S., Valent, B., and Lee, Y.-H. (2009) The ER chaperone LHS1 is involved in asexual development and Rice infection by the blast fungus *Magnaporthe oryzae*. *Plant Cell* **21**: 681–695.

Yuan, H., Blackwell, M., Mcgrath, S., George, T., Granger, S., Hawkins, J., *et al*. (2016) Morphological responses of wheat (*Triticum aestivum* L.) roots to phosphorus supply in two contrasting soils. *J Agric Sci* **154**: 98–108.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Hierarchical clustering heatmap of differentially expressed genes having an absolute value of log2 fold-change difference ≥ 4 in winter wheat cultivar NF101 at

different stages of *S. bescii* colonization under nutrient replete, N-, and P-limitation conditions. The signal ratios are shown in a red-blue colour scale, where red represents up-regulation and blue represents down-regulation. Each column namely, Full N P, N limitation, P limitation for early and late stage represents log2 fold-change value and each row represents a differentially expressed gene. The hierarchical clustering between all the differentially expressed genes were shown on the left tree.

Figure S2. qRT-PCR validation of representative genes. Bars denote mean log_2 fold change. Stacked bar denote representative gene is differentially expressed in more than one treatment.

Figure S3. Estimation of relative abundance of *S. bescii in planta*. (A) Standard curve (B) Relative abundance of *S. bescii* in wheat root across different nutrient treatments in two different stages of colonization

Supplementary Table 1. Number of differentially expressed wheat genes for all three nutrient treatments at two different stages of colonization

Supplementary Table 2. List of differentially expressed genes, for all three nutrient treatments at two different stages of colonization. Differentially expressed genes are represented as log2 transformed values. Differential expression testing was performed using DESeq2. Genes were considered significantly differentially expressed between nutrient-replete and -deplete conditions if it passed the significance threshold requiring the adjusted p-value <0.05 and an absolute value of log2 fold-change difference ≥ 2.

Supplementary Table 3. List of enriched KEGG pathways, for all three nutrients treatments at two different stages of colonization. The background number is the total number of genes in a certain pathway. The input number is the number of differentially expressed genes mapped to the indicated pathway. The Rich factor is the ratio of the number of differentially expressed genes to the total gene number in a certain pathway. FDR-corrected p-value <0.05 was considered as significantly enriched.

Supplementary Table 4. List of all the differentially expressed wheat genes induced by *S. bescii*, that plays critical role in fungal colonization, nitrogen and phosphorus metabolism.

Supplementary Table 5. List of all the primers used in the current study