A split supply of bio-organic and chemical fertilizer synergistically affects root system architecture and improves above-ground growth in pear tree (*Pyrus pyrifolia* Nakai)

Yalong Kang

Nanjing Agricultural University

Xiangrui An

Nanjing Agricultural University

Yanwei Ma

Nanjing Agricultural University

Yan Li

Hubei Engineering University

Wenli Wu

Nanjing Agricultural University

Caixia Dong (✉ cxdong@njau.edu.cn)

Nanjing Agricultural University <https://orcid.org/0000-0002-8176-3178>

Yangchun Xu

Nanjing Agricultural University

Qirong Shen

Nanjing Agricultural University

Research article

Keywords: Pear, split-root, root system architecture, bio-organic fertilizer, synergistic effect

Posted Date: February 19th, 2020

DOI: <https://doi.org/10.21203/rs.2.23996/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Root system architecture (RSA) is highly plastic, responding to nutrient availability and the heterogeneity of the soil environment. However, the linkage of root morphology to anatomy and root nutrient, and its implication for root function at the heterogeneous application of bio-organic and chemical fertilizer have not yet been defined, especially for pear trees.

Results: In this study, a split-root experiment was conducted using 1-year old 'Cuiguan' trees. No fertilizer (NF), chemical fertilizer (CF), and bio-organic fertilizer (BIO) were paired to test six combinations: NF-NF, NF-CF, NF-BIO, CF-CF, BIO-BIO, and BIO-CF. Root morphological, anatomical traits and root nutrient concentrations, and their relationships were determined. Trees receiving BIO had significantly higher lateral root numbers, activity, total lateral root length, and specific root length than trees receiving no BIO, while the effects on root tissue density were the direct opposite. Compared with CF-CF treatment, root xylem thickness, stele diameter, vessel diameter, and number of vessels all increased in response to BIO-CF treatment. Root growth was synergistically promoted in BIO-CF, with increased special root length and root nitrogen concentration, but root tissue density and the carbon:nitrogen ratio were reduced. Intriguingly, the synergistic effect resulted in greater trunk girth without sacrificing height, compared to trees receiving CF or BIO alone.

Conclusions: The combination of BIO and CF improves root traits and tree growth, suggesting that using bio-organic fertilizer as a supplement to reduce the application rate of chemical fertilizer is beneficial to orchard ecosystems.

Background

Global climate change, accelerated urbanization, and continued population growth (7.3 billion today, to an estimated 9.7 billion in 2050) pose a serious challenge to the future development of the world's agriculture, while the demand for agricultural products is expected to increase by 60–110% [1]. Modern plant breeding has resulted in significantly improved yields of all major crops, primarily by optimizing their above-ground components, the other half of the plant though, the roots, has largely been ignored [2]. Roots absorb water and nutrients from the soil and play a vital role in adapting the plant to soil environments, they are a key factor in the “second green revolution” to increase crop yields [3]. Root systems have a high level of plasticity allowing them to utilize nutrients and water in the soil despite their spatial distribution; the configuration roots take is thus called the root system architecture (RSA).

RSA is fundamental to plant productivity, but it is complex and variable making it difficult to link architecture and function [4]. RSA consists of four main parameters: growth, branching, surface area, and angle. These parameters construct a sophisticated topology and geometry in response to the spatial and temporal heterogeneity of soil nutrients. Since root systems are difficult to measure, RSA is often described using quantitative topology (mainly branching patterns) and geometric structures (inter-branch distance and branching angles) [5, 6]. Roots topologies fall between two extreme branching patterns-

dichotomous and herringbone. The herringbone architecture is expansive, thus minimizing inter-root competition, and is advantageous in soils with scarce nutrients, but has a higher C cost [7]. In contrast, the fork-like dichotomous root systems can better access diffusion-limited resources, such as phosphorus [5]. Together, the topology, branch length, branch angle, and branch density all affect nutrient absorption and retention [5, 8]. In addition to connectivity among different root segments (branch patterns), the location of a specific root segment on the larger root branching system (root orders) plays a primary role in determining the function of a single root [4]. For instance, no matter a root segment is a marginal, 1st-order root or an interior, 5th-order root can imply the deviations between a largely absorptive or transportable function and a variable longevity which ranges from days to decades [4, 9].

RSA is regulated by genetic traits, and soil physical-chemical properties such as nutrient availability and microbial populations [10]. In agricultural production, the type and quantity of fertilizer are the factors that most directly affect the soil environment, which directly affects RSA, followed by above-ground biomass and yield [11]. However, in addition to increased nitrogen content, continuous large-scale application of chemical fertilizer results in soil acidification, soil compaction, and decreased permeability. These factors cause stunting of primary roots and reduction in the number of lateral roots and branches [12].

Application of organic fertilizers such as manure, compost, and worm castings add to soil organic matter, alleviate soil acidification, and increase soil microbial activities, all of which improve crop yield [13]. Unlike chemical fertilizers, organic fertilizers promote the growth of crop roots by decomposing organic matter releasing humus. Humus influences root morphology, it induces proliferation of lateral roots and root hairs, and enhances root cell differentiation [14], which may be related to the presence of auxin in some humus [15]. Bio-organic fertilizer is an organic fertilizer with added functional microorganisms; these improve soil quality, promote plant growth, inhibit soil-borne diseases, and very importantly, are conducive to sustainable agricultural development [16, 17]. Studies on bio-organic fertilizer have largely focused on the prevention and control of soil-borne diseases [17], relief from continuous cropping [18], the composition of soil microbial communities and their functional diversity [19, 20], and aboveground biomass and yield [21]. There has been a lack of attention on the effect of bio-organic fertilizer on RSA.

Pear is the world's third largest fruit industry, and China's pear orchards account for the world's largest production area and yield [22]. The RSA of pear trees varies greatly with age, soil depth, and spatial heterogeneity of soil nutrients; the RSA of adult trees is very large and complex. Previous studies using cultivated plants and fruit trees have employed integrated excavation methods to measure RSA diameter, the number of roots, their distribution and depth, and root tissue density (RTD) [23, 24]. However, this method is difficult to use when studying the RSA of adult pear trees, therefore, studies on the RSA of pear trees have largely been on small trees or saplings.

The goal of modern intensive pear production is to sustainably and predictably produce high yields. To achieve these goals the sustainable management of soil fertility is fundamental. Long-term application of chemical fertilizer leads to a decrease in pear yield and quality [25], while the application of bio-organic

fertilizer can increase pear yield and improve fruit quality (see Fig. S1, data unpublished). The application of bio-organic fertilizer in pear orchards can be toilsome and costly, and it has proven difficult to promote its use in large orchards, but it is essential for maintaining soil health [13, 20, 21, 26]. The use of bio-organic in conjunction with chemical fertilizer has proven cost effective, but there are few studies on how the RSA of pear trees respond to soil nutrient heterogeneity caused by a bioorganic/chemical fertilizer combination.

The main purpose of this study was to investigate the effects of a BIO-CF combination on the RSA and above ground growth of pear trees. We used split-root planting boxes to grow trees half in soil supplemented with BIO and half in soil supplemented with CF. The controls were trees grown in BIO-BIO, CF-CF, and NF-NF. We analyzed the morphology, anatomy, and chemical properties of the roots under these conditions to clarify the relationship between the root system traits, soil physical-chemical properties and plant growth.

In this study we proposed the following hypotheses: 1) Bio-organic fertilizer should increase lateral root number and length, and shorten branching distance. 2) Combined application of BIO and chemical fertilizer should have a synergistic effect on the growth of pear roots, resulting in increased trunk girth.

Results

Effect of fertilizers on the physical and chemical properties of soil

The chemical fertilizer used in our study had little effect on soil pH and increased the soil C/N ratio by about 10%, while the bio-organic fertilizer added 0.3 - 0.7 pH units and increased the soil C/N ratio by about 50%. In boxes with mixed NF-fertilizer (NF-BIO, NF-CF), the addition of BIO in the right chambers increased the SOM, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and available K in the left (NF) chambers more than did the addition of CF in the right chambers. In the boxes containing BIO-CF, the soil available P and K, and C/N ratio were significantly reduced in the CF chambers compared to CF-CF boxes (Table S2).

Effect of fertilizers on pear tree growth

The height and girth of trees in NF-BIO boxes were about 24% and 22% greater, respectively, than trees in NF-NF boxes. Trees in NF-CF boxes were not significantly taller than trees in NF-NF boxes, but their trunk girth was about 14% greater ($P < 0.05$). Trees in BIO-BIO boxes were about 24% taller than trees in CF-CF boxes and their girth was greater by 14%. Trees in BIO-CF boxes were about 18% taller and 19% greater in girth than trees in the CF-CF boxes (Fig 2). These results demonstrate that the growth of the aerial parts of the pear trees were consistently greater in bio-organic fertilizer than in chemical fertilizer.

Effect of fertilizers on root morphology

The lateral roots of trees grown in the NF chambers of NF-BIO boxes were greater by all measures than were the lateral roots of trees grown in the NF chamber of NF-CF boxes (Fig 3). In boxes that contained

the same growth media in both chambers, the 2nd and 3rd lateral root numbers (LRN) accounted for the largest proportion of total LRN. In NF-NF boxes they accounted for nearly 55% of the total, in CF-CF boxes they accounted for 49% and in BIO-BIO boxes they accounted for 25% (Fig 3H). The number order of lateral roots was above 9 in BIO chambers, and below 7 in NF and CF chambers (Fig 3G). Because of the very small number of lateral roots above 6th order in any treatment, all lateral roots of 6th order and above were classified as 6th order for ease of statistical analysis (Fig 3H and I). The roots in chambers containing BIO had a significantly greater total number of lateral roots (TLRN) and greater lateral root density, and a smaller mean inter-branch distance (MID), than roots in any other media. The TLRN and lateral root density of pear trees in BIO-BIO boxes were, respectively, 2.7 and 1.8 times higher than trees in CF-CF boxes (Fig. 3A, B and F). This is due to the significantly increased proportion (up to 67 %), of 4th, 5th, and 6th order lateral roots to TLRN in the BIO-BIO boxes (Fig 3H). Compared to boxes that contained the same fertilizer in both chambers (BIO-BIO and CF-CF), in BIO-CF boxes the TLRN in BIO chambers did not change significantly, but the MID decreased by about 40%; the TLRN in the CF chambers increased by about 50%, while the MID did not change (Fig 3A and F). This is because the orders of the lateral roots (especially 2nd to 6th order) in the BIO chambers (Fig 3H and S3A-E) did not change, but were more evenly distributed on their parent roots, and the length of the lateral roots above 3rd order was increased (Fig S4B-E), while in the CF chambers the proportion of 4th and 5th order LRN to TLRN, LRL to TLRL were all increased (Fig 3H and I). This data is consistent with the increase in TLRL, total lateral root surface area (TLRS), and total lateral root volume (TLRV) (Fig 3C-E) in BIO-CF boxes, indicating that the combined application of BIO and CF had a mutually beneficial effect on root growth.

The higher the root order, the smaller the lateral root diameter (LRD). The average diameter of the 2nd lateral roots in the NF chambers of NF-BIO boxes was about 2.5 mm, and the average diameter of 2nd lateral roots in all the other media was less than 2 mm (Fig 4A), which is defined as fine roots. The mean LRD in the BIO chambers of all boxes was between 0.9 mm and 1.2 mm, while the mean LRD in CF-CF or NF-NF boxes was about 0.8 mm (Fig 4F).

Specific root length (SRL) and the ratio of root surface area to root dry biomass (RSAB) of trees in NF-BIO boxes were significantly higher than of trees in NF-NF and NF-CF boxes (ANOVA F value: 31.2, p-value <0.001) (Fig 5A, B). Root tissue density (RTD) was significantly increased in the NF chambers of NF-CF boxes, while in NF-BIO, the RTD in the NF chambers was decreased (Fig 5C). Compared to CF-CF boxes, the SRL, RSAB, and branch intensity (BI) in the BIO-BIO boxes were increased by 67%, 94% and 30%, respectively (Fig 5A, B and D), while the RTD was reduced by approximately 50% (Fig 5C). In BIO-CF boxes, the SRL and RSAB in both chambers were greater, and the RTD's were smaller than in either BIO-BIO or CF-CF media (Fig 5A, B and C).

Effects of fertilizers on root anatomy

Our results thus far indicated that a mixed application of bio-organic and chemical fertilizer had a synergistic effect on the RSA of pear trees. Therefore, to study these distinctions in root anatomy, we

focused on the RSA between CF-CF, BIO-BIO, and BIO-CF (Fig S5). Compared with CF-CF, trees grown in BIO-BIO and BIO-CF had a significantly thicker xylem, phloem, and cork layer (Fig 6A, B and C; Fig S5), as well as an increased stele:root diameter ratio (SDRD), vessel density, stele radius and vessel diameter (Fig 6D, E, F and H).

Effect of fertilizers on nutrient concentration and root activity

The root carbon, and nitrogen concentration (RCC, RNC) in BIO chambers were substantially higher than in NF or CF chambers (Fig 7A and B), while the RCC/RNC ratio in NF-NF boxes was significantly higher than in any of the other boxes (Fig 7C). Compared with NF-NF media, trees grown in BIO-BIO had significantly greater RCC and RNC, 22% and 142.3% respectively, while CF-CF grown trees had about 50% more RNC. In the NF chambers of NF-BIO boxes, the RCC and RNC were greater by 17.6% and 77% respectively, than in NF-NF boxes (Fig 7A and B).

Compared with BIO-BIO grown trees, the roots in the BIO chambers of BIO-CF grown trees had about 10% and 5% greater RCC and RNC respectively. Compared with CF-CF grown trees, the roots in the CF chambers of BIO-CF trees had about 36% greater RNC, there was no significant change in RCC (Fig 7A and B). Trees grown in BIO had significantly greater root activity than trees not treated with BIO, and the root activity in the CF chambers of BIO-CF grown trees was about 50% higher than in CF-CF grown trees ($P < 0.05$) (Fig 7D).

Effect of soil properties on RSA and root nutrient content

Figure 8 illustrates the correlation between the properties of our test soil/fertilizer mixes and the RSA of the pear trees grown in them. Soil pH and SOM significantly promoted RA, SRL, TLRL, TLRN, and RNC. NO_3^- -N and NH_4^+ -N were positively correlated with RA, SRL, and RNC, but only NO_3^- -N obviously promoted BI and RSAB, while NH_4^+ -N significantly promoted TLRL and TLRN. AK was correlated with increased LRN and higher RA, which may be related to SOM.

Linear regression analysis of the relationships between root traits and root nutrient content

Total lateral root length (TLRL) and specific root length (SRL) increased with increasing mean lateral root diameter (MRD), while root tissue density (RTD) decreased with increasing MRD (Fig 9A-C). SRL increased with increasing TLRN, while RTD decreased with increasing TLRN (Fig 9D-E), and the larger of the SRL, the smaller of the RTD (Fig 9F). Root nitrogen concentration (RNC) increased with increasing MRD (Fig 9G). RCC/RNC decreased with increasing SRL and increased with increasing RTD (Fig 9H-I). In summary, we found a close correlation between the RSA parameters and the root nutrient content of the pear trees, together these factors determined the nutrient absorption efficiency of the roots.

Redundancy analysis of RSA traits and soil factors

By RDA analysis we found that RSA parameters and root carbon and nitrogen content responded differently to soil physical-chemical factors induced by fertilization (BIO and CF) (Fig 10). RDA1 and

RDA2 accounted for 46.1% and 5.9% of the total variation, respectively. Bio-organic fertilizer (NF-BIO, BIO-BIO and BIO-CF), increased soil pH and SOM promoting TLRN, TLRL, SRL, and enhanced root activities and root nutrient content, and reduced RTD and RCC/RNC. Additionally, bio-organic fertilizer improved soil NO_3^- -N and NH_4^+ -N levels which led to significantly increased TLRN, SRL, RA and RNC of pear roots, but had little effect on RTD and MID (Fig 8 and 10, table S1).

Discussion

In this study we found that between the chambers of split root boxes containing homogenous media (CF-CF, BIO-BIO, NF-NF), there were no significant differences in the morphology or anatomical structure of roots of pear trees (Fig 3, 6 and S5), demonstrating that the split-root boxes and systematic error were negligible in analyzing the influence of soil nutrient heterogeneity on RSA; in other words, the differences in RSA were due to the heterogeneous experimental treatments. in between chambers

BIO-CF had a significant synergistic effect on the growth of lateral roots

Plants must perceive physical and chemical changes in soil nutrient heterogeneity to optimize their root architecture system (RSA) [34], thereinto, lateral root number (LRN), lateral root density (LRD) and inter-branching distance are all parameters that reflect the high plasticity of root systems, and they are all sensitive to nutrient heterogeneity [35, 36]. Lateral roots are a key factor determining the RSA of pear trees due to their taproot system. We found that bio-organic fertilizer (BIO) significantly increased the number, length and density of lateral roots of pear trees, and reduced inter-branching distance (Fig 3A -C and F), supporting the first hypothesis proposed in this paper. Different orders of lateral roots have different functions; lateral roots less than 2 mm in diameter are the most activate part of the root system [37], playing a key role in absorbing soil nutrients and water [9, 27]. We found that while the diameter of 2nd order and above lateral roots of trees grown in all media was 2 mm or less (Fig 4), the number and length of lateral roots in BIO-CF boxes was significantly higher than that of CF-CF boxes, indicating that the bio-organic/chemical fertilizer combination was particularly conducive to allowing pear roots to occupy a large soil space and thereby improve nutrient absorption efficiency. In addition, lateral roots grown in BIO reached about 10 orders, lateral roots grown in CF-treated were below 7 orders (Fig 3G). Khan et al. [38] argued that the application of organic fertilizer significantly increased the number of lateral roots of oats, but did not focus on the maximum order that can be reached by lateral roots.

In order to study the mechanism of plant response to nutrient heterogeneity, the split-root is the most effective method [39, 40]. Compared to the roots growing in boxes with the same media in each chamber, plants grown in boxes with a different media in each chamber can only absorb 75% of the nutrients in their own chamber and the growth of the root is asymmetrical [41]. This is consistent with the differences we saw in the growth of the pear roots treated with NF-BIO, NF-CF, and BIO-CF (Fig 3). The heterogeneity of soil nutrients stimulates long-distance signal transduction in plants, regulating hormones such as abscisic acid (ABA), growth hormone (such as IAA), and cytokinin (CK), which affect lateral root growth, and the redistribution of photosynthetic products (for example, amino acids). Together, these act to

optimize the RSA of plants, allowing them to better exploit the soil environment [42, 43]. We found that application of BIO in one chamber of split root boxes significantly increased the number and length of lateral roots in the neighboring NF chamber, while the analogous application of CF has no significant effect on the number or length of lateral roots in the neighboring NF chamber (Fig 3A and C). Interestingly, in BIO-CF boxes the TLRL, TLRS, and TLRV of pear roots were increased in both chambers over the roots of trees in BIO-BIO or CF-CF (Fig 3C-E), demonstrating an obvious synergistic effect. This BIO-CF heterogeneity may cause an alteration in the distribution of root exudates, promoting root growth toward the patch of elevated available soil nutrients [44-46]. Further research is however needed to determine the changes in the composition and distribution of the root exudates and their effects on the organization of rhizospheric microbes.

More than 80% of soil microbes can synthesize IAA, and tryptophan is considered the core substrate for bacterial synthesis of IAA [47, 48]. High concentrations of IAA stimulate the formation and growth of lateral roots, thus increasing their surface area, which is favorable for the absorption of soil nutrients [49]. Bio-organic fertilizer contains *Bacillus amyloliquefaciens* SQR9 which enriches the rhizosphere and colonizes root surfaces (Fig. S6); SQR9 contains the functional gene *dhaS*, which encodes indole-3-pyruvate decarboxylase (IPDC) that in turn leads to production of IAA by the indole-3-pyruvic acid (IPyA) pathway [47, 48], thereby stimulating the formation and growth of lateral roots. Chemical fertilizer delivers a large amount of available nutrients over a short time frame, this high nutrient availability may result in the increased content of tryptophan in the photosynthetic products distributed to roots. In this way, beneficial microorganisms may be recruited, enriching the rhizosphere and boosting IAA production, thereby stimulating lateral root growth. We found that BIO-CF had a synergistic effect, obvious in increased trunk girth (about 19%) without a concomitant sacrifice to tree height (Fig 2 and Fig 11); this supports our second hypothesis.

BIO-CF resulted in increased SRL and reduced RTD

Root characteristics, such as RTD and SRL, reflect their role in maximizing access to and use of limited soil nutrients [50]. RTD increases as soil nutrient availability decreases [51], this tendency is associated with slower root growth and longer lifespans. Greater SRL indicates root systems have built more root length per unit dry-mass, generally these roots have higher rates nutrient and water uptake but shorter lifespans than low SRL plants [52]. In contrast, Kramer-Walter et al. [50] argued that low soil nutrients would result in increased SRL, due to root systems needing to explore more soil volume to obtain nutrients. In our study, the application of BIO in one chamber of a split root box resulted in increased SRL and decreased RTD of lateral roots in the other chamber of the split root box (Fig 5A, C and Fig 11). This may be because when the pear tree roots sensed a heterogeneous soil environment, the carbon input per unit of root length increased, causing a change in and redistribution of root exudates (Fig 7A), thereby prompting roots to spread over a large soil space. Another important finding from this study is that the BIO-CF combination resulted in increased SRL and reduced the RTD on roots in each chamber (Fig 5A, C and Fig 11). This may indicate that the lifespan of lateral roots is shortened, thereby increasing nutrient absorption efficiency [53]. Many studies suggested an intimate correlation between fine roots diameter

and lifespan [54, 55]. However, understanding how the relationship between fine roots diameter and lifespan of pear trees is affected by the BIO-CF heterogeneity is also a meaningful research priority.

BIO-CF resulted in increased average vessel diameter, number of vessels and their density of lateral roots

To determine how the RSA of pear trees responds to heterogeneous fertilization (BIO-CF), we used homogeneous fertilization (CF-CF and BIO-BIO) controls, and 4th order lateral roots to analyze the relationship between anatomical structure and fertilization strategy. The root samples collected in this study were mature lateral roots, (collected 120 DAB), meaning their outermost cell structure was a cork layer (Fig S5). This is similar to what Wang *et al.* [56] reported for 2-year-old *Pinus tabulaeformis*, from which more than 87% of the 3rd order lateral roots were lignified, as were all 4th order and overtop lateral roots. Vessel diameter and vessel density are key parameters determining water and nutrient transport efficiency [9]. El-Nagdy et al. [57] reported that bio-organic fertilizer mix 50% (w / w) chemical fertilizer resulted in significantly increased the main stem diameter, epidermal thickness, cortex, secondary xylem, and secondary phloem of flax. Anatomical studies of beet roots have shown that use of bio-organic fertilizers resulted in increased thickness of the root growth ring and the average diameter of xylem vessels [58]. We found that the application of BIO or BIO-CF resulted in increased xylem and phloem thickness, average vessel diameter, number of vessels and their density in pear roots (Fig 6 and Fig 11). These factors improve the absorption efficiency of nutrients and water by lateral roots and are consistent with the morphology of lateral roots in BIO and BIO-CF described above.

BIO-CF resulted in increased RNC and reduced RCC/RNC

Generally, because of the rapid turnover of fine roots, the usually have a high N concentration and low C/N ratio [59]. Our results showed that in BIO chambers, RNC and root activity (RA) was higher than in NF and CF chambers (Fig 6B and D), while RCC/RNC was lower (Fig 7C). These data indicate that BIO not only improves RA, promoting the absorption of soil available nutrients, but also may accelerate the rate of root turnover. This corresponded with our earlier observations that BIO increased SRL but reduced RTD (Fig 5A and C). The application of BIO in one chamber may also promote the absorption of soil nutrients and turnover rate of fine roots in the companion chamber, especially a CF companion chamber (Fig 7 and Fig 11). In low nutrient content conditions (NF-NF), RNC was also low (Fig 7B). The findings of this study are consistent with those reported by Kramer-Walter and Laughlin [60].

Conclusions

In our study, use of bio-organic fertilizer resulted in increased LRN, LRL, SRL, and RA, and decreased RTD, all of which enhance the nutrient absorption capacity of roots. Bio-organic fertilizer applied in one chamber of the split root boxes promoted root growth in the unfertilized companion chambers; analogous application of CF did not have these effects. The growth of roots in each chamber of BIO-CF boxes was not only significantly enhanced over the growth of roots in boxes containing BIO-BIO and CF-CF, they also had higher nitrogen content, a lower carbon-nitrogen ratio, greater xylem and phloem thickness, stele

radius, and number of vessels. These factors increase the capacity for the absorption of soil nutrients, and demonstrated the synergistic effect of BIO-CF. The pear trees grown in BIO-CF boxes had greater trunk girth, without a sacrifice in height, than trees grown in BIO-BIO or CF-CF. Therefore, combinations of bio-organic and chemical fertilizer may be a valuable means to ensure the sustainable development of pear orchards. These data also offer broad support for China and the global agricultural enterprise for reducing the use of chemical fertilizer, while still benefiting from a robust and profitable yield.

Methods

Substrates and materials

In October of 2017, approximately 3000 kg of surface soil, 0 to 30 cm deep, was collected from the pear garden site of the Lvyuan Fruit Industry Co., Ltd., Jianning County, Sanming City, Fujian Province, China (34°04' N, 108° 10' E). The soil type is red loam and the texture is clay, the field water holding capacity is 33.8%, soil organic matter content is 15.70 g kg⁻¹, total nitrogen content is 2.08 g kg⁻¹, available potassium content is 184.5 mg kg⁻¹, available phosphorus content is 45.8 mg kg⁻¹, and the pH is 5.33. The collected soil was naturally air-dried and passed through a 5 mm sieve before use. From January to November 2018, we carried out a potted split-root test at the Nanjing Agricultural University Test Base (31°36' N, 119°10' E), Baima Town, Lishui District, Nanjing, Jiangsu Province. The climate type at test site is the transition zone from north subtropical to mid-subtropical. The climate is mild and humid, the annual average for temperature is 15.4 °C, sunshine is 2240 h, and rainfall is 1087.4 mm, the frost-free period is 237 d. For the monthly climatic conditions during the test period see Fig. 1.

Split-root boxes and experimental design

Brown plastic planting boxes, 39 x 39 x 45 cm (l x w x h), were used for the split-root test, each box was divided into two 39 x 19 x 45 cm (l x w x h) chambers, the planting depth was 40 cm. The base plate (39 x 39 x 3 cm) of each box was black plastic, with a 10 mm diameter water outlet on each of the four sides. Four hollow poles (21 cm x 2 mm) were seeded into the base plate and four black mesh water-repellent plastic plates with many 2 mm diameter filter holes were slid into place between the poles, forming the sides of the box, each seam was then sealed with strips of black plastic. The box was then divided into two equal chambers with a white hard plastic plate 39 x 38 x 0.3 cm, (l x w x h), and marked with L (left) and R (right). The interface between the partition and the panel was sealed with waterproof 580 glass glue, and was allowed to dry naturally. In order to facilitate the fixation of the root systems, a 3 x 3 cm² notch was cut in the middle of the top of the white plate (Fig. S2).

On January 25, 2018 approximately 25 kg of air-dried soil (bulk density 0.84 g cm⁻³) was loaded into the compartments of each planting box and irrigated until the soil water content was about 30% (vol). 1-year old dormant 'Cuiguan' pear trees with two fork-shaped primary roots, in this experiment, according to the root order nomenclature by Barczi et al. [27], we treated them as the 1st order roots, that were nearly identical in length were selected and planted. Each rootstock was *Pyrus betulaefolia* and the height above

the base was approximately 60 cm. Roots were distributed as evenly as possible into the two chambers, then each box was wrapped in black plastic grass cloth to prevent moisture evaporation and weed growth. The following six treatments were set up for the split-root test: no fertilizer in either chamber (NF-NF), no fertilizer in the L chamber-chemical fertilizer in the R chamber (NF-CF), no fertilizer in the L chamber-bio-organic fertilizer in the R chamber (NF-BIO), chemical fertilizer in both chambers (CF-CF), bio-organic fertilizer in both chambers (BIO-BIO), bio-organic fertilizer in the L chamber and chemical fertilizer in the R chamber (BIO-CF); there were six replicates per treatment. The bio-organic fertilizer was purchased from Jiangsu Xinli Bio-fertilizer Engineering Center Co., Ltd. This product was a mix of solid decomposed organic fertilizer and agricultural amino acids (6:4 dry weight) inoculated with *Bacillus amyloliquefaciens* SQR9. *B. amyloliquefaciens* SQR9 colonies per gram of fertilizer were 1×10^9 CFU g⁻¹ dry weight. The composition of this fertilizer was N 4.77%, P₂O₅ 2.26%, and K₂O 1.00%. Each BIO chamber received 500 g of fertilizer as a one-time base treatment, this amounted to 2% (w/w) to soil. Chemical fertilizer consisted of analytically pure urea (N 46%), potassium sulfate (K₂O 50%) and superphosphate (P₂O₅ 15%). 70% of the urea and potassium chloride and 100% of the superphosphate were added to the CF chambers as a base fertilizer on Jan 25, 2018. The remaining of urea and potassium chloride was applied as a top dressing on June 20, 2018. Each CF chamber received equal amounts of N, P, and K (23.8 g N, 13.3 g P₂O₅, 23.8 g K₂O).

These fertilizers were granular solids which were fully dissolved in 2 L water before application, and were uniformly applied to the chambers in combination with irrigation. The trial period began on March 20 (germination stage) and ended on November 10 (abscission period). During this time an automatic drip irrigation system was used to supply the water to the trees, the flow rate was 12.5 ml min⁻¹. The weekly irrigation schedule was 18:00 ~ 20:00 on Wednesdays and Saturdays, except for July to August, when it was 18:00 ~ 20:00 on Tuesdays, Thursdays, and Saturdays.

Sampling and measurements

210 days after budding (DAB), plant heights from the base of the trees to the highest apical bud were measured with a tapeline, and trunk girths were measured with a Vernier caliper 15 cm from the base of the trees. From each treatment group, three plant boxes were randomly selected, and the root systems were phenotyped using Shovelomics [28]. A black marking pen was used to mark on the “L” side of each trunk, about 50 cm from the base, to distinguish the root chambers. Roots were gently extracted from the box chambers and were cleaned of soil by gentle shaking, sterile tweezers, then slow agitation while immersed in a 60 cm diameter plastic bucket of water. Finally, the root systems were gently rinsed with tap water until no soil remained.

The number of second order lateral roots were counted manually, then three second order lateral roots (2nd LR) about 20 cm in length, were randomly selected from each side (L and R) of each sample, and cut away from the parent roots using a sterilized garden shear. These roots were placed horizontally on a plastic plate covered with a black photography cloth, the entangled and overlapping roots were separated using a sterile tweezer then photographed with a Canon EOS 750D Digital SLR camera (EF - S 18 - 135

mm f / 3.5 - 5.6 IS STM lens, 24.2 million) fixed at a vertical height of 60 cm above the samples. All images were saved for subsequent RSA analysis. After photo documentation, all roots were separately placed in a bubble envelope and dried at 70 °C for 72 h and then weighed. The specific root length (SRL m g^{-1} , the ratio of root length to dry mass of fine roots), root tissue density (RTD g cm^{-3} , root dry mass divided by fresh root volume), and the ratio of root surface area to root dry biomass (RSAB, $\text{cm}^2 \text{g}^{-1}$) were calculated. Fine roots (diameter < 2 mm) were smashed in a SPEX 8000-D hybrid grinder (SPEX, Edison, NJ, USA) to analyze the root carbon concentration (RCC) and root nitrogen concentration (RNC). Ten fourth lateral roots (4th LR) were also randomly selected from each side of each sample, cut into 1 cm segments starting from the root apex, then fixed for 48 h in FAA fixative solution (90 ml of 50% ethanol, 5 ml of 100% glacial acetic acid and 5 ml of 37% formaldehyde) for anatomical structure analysis.

Root morphology

Semi-automatic SmartRoot (<https://smartroot.github.io>) [29, 30] combined with ImageJ 1.46R (<http://imagej.nih.gov/ij/download.html>) were used to analyze the lateral root images for total lateral root length (TLRL), total lateral root surface area (TLRA), total lateral root volume (TLRV), total number of lateral roots (TLRN), mean lateral root diameter (MRD), mean branching angle (MRA), and mean inter-branch density (MID), maximum order of lateral roots (MOLR), and branching intensity (BI).

Root anatomy

The FAA fixed root segments were stained with 2% saffron green then dehydrated in a series of increasing ethanol concentrations 75%, 85%, 90%, 95%, 100%, then alcohol/benzene, xylene, and then embedded in 65 °C paraffin. From each sample 3 to 5 root sections, about 8 μm thick, were cut with a RM2016 microtome (Shanghai Leica Instrument Co., Ltd.) then embedded in Technovit 7100 resin (Heraeus Kulzer, Germany). From each sample, three slides were randomly chosen for observation. Slides were photographed using a DM21-J1200 optical microscope and ScopelImage 9.0 software. ImageJ 1.46R was used to measure cork layer thickness, xylem thickness, phloem thickness, vessel diameter, stele radius, number of vessels in each stele, total cross-sectional area of the xylem (CSAX), and the ratio of stele diameter to root diameter (SDRD). Representative cross-sectional images of these 4th lateral roots are shown in Fig. S5.

Root chemical analysis and activity

Root carbon and nitrogen concentrations were measured using a Macro Elemental Analyzer (vario MACRO, Elementar Co., Hanau, Germany). Root activity was measured using the TTC (triphenyl tetrazolium chloride) method described in Chen [31], activity was expressed as the deoxidization ability ($\text{mg g}^{-1} \text{FW h}^{-1}$).

Soil sampling and chemical analysis

Soil samples from roots were collected 210 days after budding (DAB). Specifically, approximately 100 g of soil was shaken from the roots in each chamber, air dried, and then passed through a 2 mm sieve to remove impurities such as stones, glass, and residual roots. An acidometer (MT-5000, Shanghai, China) was used to measure soil pH. A flow injection auto-analyzer (AA3, Seal Co., Germany) was used to determine soil ammonium ($\text{NH}_4^+\text{-N}$) and nitrate ($\text{NO}_3^-\text{-N}$) concentrations. Total soil N concentrations were determined using an elemental analyzer (Vario Macro, Elementar Analysensysteme, Germany). Soil organic matter content (SOM) was determined by the chromic acid titration method according to Bao [32]. Ammonium acetate was used to extract soil available K (AK), which was then quantified using flame photometry. Sodium bicarbonate was used to extract soil available P (AP), which was then quantified using the molybdenum blue method Bao [32].

Statistical analyses

One-way ANOVA was used to identify the effects of chemical and bio-organic fertilizer on root morphology, anatomy, and chemical composition. The difference in significance between treatments was determined by Fisher's LSD analysis ($P < 0.05$). Pearson's correlation was used to determine the relationships among RSA parameters, anatomical characteristics, roots chemical concentrations, and soil physicochemical traits using R v.3.1.3 software. Linear regression analysis was used to describe the relationship between the key RSA parameters of pear trees. Root traits and environmental factors were completed with the CANOCO 4.5 software package using R v.3.1.3 software [33]. Redundancy analyses (RDA) were used to test the interrelationships of root traits, and their responses to soil nutrient following the different fertilization. All statistical analyses were performed using the IBM SPSS 20.0 software. Visualization of all data was done in the Origin 2019b software.

Supplementary Information

Additional file 1:

Table S1. Fertilization protocol

Table S2. Properties of the soils from the split-root chambers

Figure S1. Effects of different fertilizers on yield and SSC of pear.

Figure S2. Planting box details.

Figure S3. Differences in lateral root number based on fertilizer composition.

Figure S4. Differences in lateral root lengths based on fertilizer composition.

Figure S5. Anatomy of pear roots.

Figure S6. Confocal microscopy of GFP-tagged *Bacillus amyloliquefaciens* SQR9 colonization on pear roots.

Abbreviations

RSA: Root system architecture; DAB: Days after budding; SRL: Specific root length; RTD: Root tissue density; RSAB: Ratio of root surface area to root dry biomass; TLRL: Total lateral root length; TLRA: Total lateral root surface area; TLRV: Total Lateral root volume; TLRN: Total number of lateral roots; MRD: Mean lateral root diameter; MRA: Mean branching angle; MID: Mean inter-branch density; MOLR: Maximum order of lateral roots; BI: Branching intensity; CSAX: Cross-sectional area of the xylem; SDRD: Ratio of stele diameter to root diameter; RCC: Root carbon concentration; RNC: Root nitrogen concentration; SOM: Soil organic matter content; RDA: Redundancy analyses; IPDC: Indole-3-pyruvate decarboxylase; IPyA: Indole-3-pyruvic acid; ABA: Abscissic acid; CK: Cytokinin; IAA: Indole-3-acetic acid.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

Funding

The study was financially supported by Pear Modern Agro-industry Technology Research System (CARS-28-10) and by the Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX18_0680).

Authors' contributions

YK wrote the manuscript; YK, XA and YM performed the experiments and analyzed the data; YK, XA, YL and WW collected the samples; CD, YX and QS contributed reagents, instruments and designed the experiment. All authors reviewed and approved the final manuscript.

Acknowledgments

The authors are grateful to Professor Sheng Qiang, Nanjing Agricultural University, for help with confirming the anatomical structure of pear roots in this paper.

Author details

1. Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, National Engineering Research Center for Organic-based Fertilizers, Jiangsu Collaborative Innovation Center for Solid Organic Waster Resource Utilization, Nanjing Agricultural University, Nanjing 210095, China
2. Hubei Key Laboratory of Quality Control of Characteristic Fruits and Vegetables, Hubei Engineering University, Xiaogan 432000, China

References

1. Long SP, Marshall-Colon A, Zhu X-G: **Meeting the global food demand of the future by engineering crop photosynthesis and yield potential.** *Cell* 2015, **161**(1):56-66.
2. Voss-Fels KP, Snowden RJ, Hickey LT: **Designer roots for future crops.** *Trends Plant Sci* 2018, **23**(11):957-960.
3. Lynch JP: **Roots of the second green revolution.** *Aust J Bot* 2007, **55**(5):493-512.
4. Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL: **Fine root architecture of nine North American trees.** *Ecol Monog* 2002, **72**(2):293-309.
5. Fitter A: **An architectural approach to the comparative ecology of plant root systems.** *New Phytol* 1987, **106**:61-77.
6. Lynch J: **Root architecture and plant productivity.** *Plant Physiol* 1995, **109**(1):7.
7. Fitter A: **The topology and geometry of plant root systems: influence of watering rate on root system topology in *Trifolium pratense*.** *Ann Bot* 1986, **58**(1):91-101.
8. Arredondo J, Johnson D: **Root architecture and biomass allocation of three range grasses in response to nonuniform supply of nutrients and shoot defoliation.** *New Phytol* 1999, **143**(2):373-385.
9. Guo D, Xia M, Wei X, Chang W, Liu Y, Wang Z: **Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species.** *New Phytol* 2008, **180**(3):673-683.
10. Rich SM, Watt M: **Soil conditions and cereal root system architecture: review and considerations for linking Darwin and Weaver.** *J Exp Bot* 2013, **64**(5):1193-1208.
11. Zhao XH, Yu HQ, Wen J, Wang XG, Du Q, Wang J, Wang Q: **Response of root morphology, physiology and endogenous hormones in maize (*Zea mays* L.) to potassium deficiency.** *J Integr Agr* 2016, **15**(4):785-794.
12. Tian Q, Chen F, Liu J, Zhang F, Mi G: **Inhibition of maize root growth by high nitrate supply is correlated with reduced IAA levels in roots.** *J Plant Physiol* 2008, **165**(9):942-951.

13. Pathma J, Sakthivel N: **Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential.** *SpringerPlus* 2012, **1**(1):26.
14. Canellas LP, Olivares FL, Okorokova-Façanha AL, Façanha AR: **Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H⁺-ATPase activity in maize roots.** *Plant Physiol* 2002, **130**(4):1951-1957.
15. Jindo K, Martim SA, Navarro EC, Pérez-Alfocea F, Hernandez T, Garcia C, Aguiar NO, Canellas LP: **Root growth promotion by humic acids from composted and non-composted urban organic wastes.** *Plant Soil* 2012, **353**(1-2):209-220.
16. Wu Y, Zhao C, Farmer J, Sun J: **Effects of bio-organic fertilizer on pepper growth and Fusarium wilt biocontrol.** *Sci Hortic* 2015, **193**:114-120.
17. Zhang N, Pan R, Shen Y, Yuan J, Wang L, Luo X, Raza W, Ling N, Huang Q, Shen Q: **Development of a novel bio-organic fertilizer for plant growth promotion and suppression of rhizome rot in ginger.** *Biol Control* 2017, **114**:97-105.
18. Li L, Ma J, Ibekwe AM, Wang Q, Yang C-H: **Influence of *Bacillus subtilis* B068150 on cucumber rhizosphere microbial composition as a plant protective agent.** *Plant Soil* 2018, **429**(1-2):519-531.
19. Chen Y, Jiang Z, Wu D, Wang H, Li J, Bi M, Zhang Y: **Development of a novel bio-organic fertilizer for the removal of atrazine in soil.** *J Environ Manage* 2019, **233**:553-560.
20. Qiao C, Penton CR, Xiong W, Liu C, Wang R, Liu Z, Xu X, Li R, Shen Q: **Reshaping the rhizosphere microbiome by bio-organic amendment to enhance crop yield in a maize-cabbage rotation system.** *Appl Soil Ecol* 2019.
21. Zhang Y, Gao X, Shen Z, Zhu C, Jiao Z, Li R, Shen Q: **Pre-colonization of PGPR triggers rhizosphere microbiota succession associated with crop yield enhancement.** *Plant Soil* 2019:1-15.
22. Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H *et al*: **The genome of the pear (*Pyrus bretschneideri* Rehd.).** *Genome Res* 2013, **23**(2):396-408.
23. Shao H, Shi D, Shi W, Ban X, Chen Y, Ren W, Chen F, Mi G: **Genotypic difference in the plasticity of root system architecture of field-grown maize in response to plant density.** *Plant Soil* 2019:1-17.
24. Zanetti C, Vennetier M, Mériaux P, Provansal M: **Plasticity of tree root system structure in contrasting soil materials and environmental conditions.** *Plant Soil* 2015, **387**(1-2):21-35.
25. Khan S, Godara R, Malik R: **Influence of nitrogen & FYM application on quality of pear (*Pyrus pyrifolia* Nakai).** *Indian J Ecol* 2017, **44**(4):266-268.
26. Li R, Tao R, Ling N, Chu G: **Chemical, organic and bio-fertilizer management practices effect on soil physicochemical property and antagonistic bacteria abundance of a cotton field: implications for soil biological quality.** *Soil Tillage Res* 2017, **167**:30-38.
27. Barczy J-F, Rey H, Griffon S, Jourdan C: **DigR: a generic model and its open source simulation software to mimic three-dimensional root-system architecture diversity.** *Ann Bot* 2018, **121**(5):1089-1104.

28. Trachsel S, Kaeppler SM, Brown KM, Lynch JP: **Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field.** *Plant Soil* 2011, **341**(1):75-87.
29. Lobet G, Pagès L, Draye X: **A novel image-analysis toolbox enabling quantitative analysis of root system architecture.** *Plant Physiol* 2011, **157**(1):29-39.
30. Mathieu L, Lobet G, Tocquin P, Périlleux C: **"Rhizoponics": a novel hydroponic rhizotron for root system analyses on mature *Arabidopsis thaliana* plants.** *Plant Methods* 2015, **11**(1):3.
31. Chen C: **Measurement of plant root activity (TTC).** *Principle and Technology of Plant Physiological and Biochemical Experiments* 2003:119-120.
32. Bao S: **Soil agrochemical analysis.** *Chinese) China Agricultural Press, Beijing* 2000:25-114.
33. Ter Braak CJ, Smilauer P: **CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5).** In.: www.canoco.com; 2002.
34. Rellán-Alvarez R, Lobet G, Dinneny JR: **Environmental control of root system biology.** *Annu Rev Plant Biol* 2016, **67**:619-642.
35. Morris EC, Griffiths M, Golebiowska A, Mairhofer S, Burr-Hersey J, Goh T, von Wangenheim D, Atkinson B, Sturrock CJ, Lynch JP: **Shaping 3D root system architecture.** *Curr Biol* 2017, **27**(17):R919-R930.
36. Tian H, De Smet I, Ding Z: **Shaping a root system: regulating lateral versus primary root growth.** *Trends Plant Sci* 2014, **19**(7):426-431.
37. Baldi E, Wells CE, Marangoni B: **Organic fertilization leads to increased peach root production and lifespan.** *Tree Physiol* 2010, **30**: 1373-1382
38. Khan T, Nadeem F, Gao Y, Yang Y, Wang X, Zeng Z, Hu Y: **A larger root system in oat (*avena nuda* L.) is coupled with enhanced biomass accumulation and hormonal alterations under low nitrogen.** *Appl Ecol Env Res* 2019, **17**(2):4631-4653.
39. Gao W, Blaser SR, Schlüter S, Shen J, Vetterlein D: **Effect of localised phosphorus application on root growth and soil nutrient dynamics in situ—comparison of maize (*Zea mays*) and faba bean (*Vicia faba*) at the seedling stage.** *Plant Soil* 2019:1-15.
40. Irving LJ, Vaughan JK, Ong G, Schwier N, Hama T, Cameron DD: **Differential carbon allocation to nitrogen-rich patches in *Poa annua* precedes root proliferation but has no immediate benefit to N uptake.** *J Plant Physiol* 2019, **234**:54-59.
41. Gile P, Carrero J: **Assimilation of nitrogen, phosphorus, and potassium by corn when nutrient salts are confined to different roots.** *J Agric Res* 1921, **21**(8).
42. Korver RA, Koevoets IT, Testerink C: **Out of shape during stress: A key role for auxin.** *Trends in plant science* 2018, **23**(9):783-793.
43. van den Berg T, Korver RA, Testerink C, ten Tusscher KH: **Modeling halotropism: a key role for root tip architecture and reflux loop remodeling in redistributing auxin.** *Development* 2016, **143**(18):3350-3362.

44. Giehl RF, Lima JE, von Wirén N: **Regulatory components involved in altering lateral root development in response to localized iron: Evidence for natural genetic variation.** *Plant Signaling Behav* 2012, **7**(7):711-713.
45. Gruber BD, Giehl RF, Friedel S, von Wirén N: **Plasticity of the Arabidopsis root system under nutrient deficiencies.** *Plant physiol* 2013, **163**(1):161-179.
46. Notaguchi M, Okamoto S: **Dynamics of long-distance signaling via plant vascular tissues.** *Front Plant Sci* 2015, **6**:161.
47. Shao J, Li S, Zhang N, Cui X, Zhou X, Zhang G, Shen Q, Zhang R: **Analysis and cloning of the synthetic pathway of the phytohormone indole-3-acetic acid in the plant-beneficial *Bacillus amyloliquefaciens* SQR9.** *Microb Cell Fact* 2015, **14**(1):130.
48. Shao J, Xu Z, Zhang N, Shen Q, Zhang R: **Contribution of indole-3-acetic acid in the plant growth promotion by the rhizospheric strain *Bacillus amyloliquefaciens* SQR9.** *Biol Fert Soils* 2015, **51**(3):321-330.
49. Vejan P, Abdullah R, Khadiran T, Ismail S, Nasrulhaq Boyce A: **Role of plant growth promoting rhizobacteria in agricultural sustainability—a review.** *Molecules* 2016, **21**(5):573.
50. Kramer-Walter KR, Bellingham PJ, Millar TR, Smissen RD, Richardson SJ, Laughlin DC: **Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum.** *J Ecol* 2016, **104**(5):1299-1310.
51. Ryser P, Lambers H: **Root and leaf attributes accounting for the performance of fast-and slow-growing grasses at different nutrient supply.** *Plant Soil* 1995, **170**(2):251-265.
52. Laliberté E, Lambers H, Burgess TI, Wright SJ: **Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands.** *New Phytol* 2015, **206**(2):507-521.
53. Eissenstat D, Wells C, Yanai R, Whitbeck J: **Building roots in a changing environment: implications for root longevity.** *New Phytol* 2000, **147**(1):33-42.
54. Adams TS, McCormack ML, Eissenstat DM: **Foraging strategies in trees of different root morphology: the role of root lifespan.** *Tree Physiol* 2013, **33**(9):940-948.
55. Weemstra M, Mommer L, Visser EJW, van Ruijven J, Kuyper TW, Mohren GMJ, Sterck FJ: **Towards a multidimensional root trait framework: a tree root review.** *New Phytol* 2016, **211**(4):1159-1169.
56. Wang G, Liu F, Xue S: **Nitrogen addition enhanced water uptake by affecting fine root morphology and coarse root anatomy of Chinese pine seedlings.** *Plant Soil* 2017, **418**(1-2):177-189.
57. El-Nagdy G, Nassar DM, El-Kady EA, El-Yamanee GS: **Response of flax plant (*Linum usitatissimum* L.) to treatments with mineral and bio-fertilizers from nitrogen and phosphorus.** *J Am Sci* 2010, **6**(10):207-217.
58. Agamy R, Hashem M, Alamri S: **Effect of soil amendment with yeasts as bio-fertilizers on the growth and productivity of sugar beet.** *Afr J Agric Res* 2013, **8**(1):46-56.

59. Zhuang L, Yang W, Wu F, Tan B, Zhang L, Yang K, He R, Li Z, Xu Z: **Diameter-related variations in root decomposition of three common subalpine tree species in southwestern China.** *Geoderma* 2018, **311**:1-8.

60. Kramer-Walter KR, Laughlin DC: **Root nutrient concentration and biomass allocation are more plastic than morphological traits in response to nutrient limitation.** *Plant Soil* 2017, **416**(1-2):539-550.

Figures

Figure 1

Weather conditions at the experimental site, January December 2018. (A) Temperature: Mean monthly, mean maximum, and mean minimum. (B) Rainfall: Mean monthly rainfall, mean number of rain days, and mean relative humidity.

Figure 2

Effect of fertilizers on pear trees (A) height and (B) girth. Measurements were taken 210 days after budding (DAB). Error bars represent 1SE of the mean ($n = 4$). Significant differences are indicated by different lowercase letters at $P < 0.05$.

Figure 3

RSA traits (A) Total lateral root number, (B) lateral root density, (C) total lateral root length, (D) total lateral root surface, and (E) total lateral root volume. (F) Mean interbranch distance (MID). (G) Maximum order of lateral roots. (H) For each order of lateral root, ratio lateral root number (LRN) to total LRN (TLRN) and (I) for each order of lateral root, ratio of lateral root length (LRL) to TLRL. Error bars represent 1 SE of the mean (n = 9). † means significant differences between the CF-CF and BIO-BIO treatments at P < 0.05, § means significant differences among the twelve chambers of the six treatments at P < 0.05, * means significant differences between the left and right chambers in boxes containing the same media in both chambers at P < 0.05.

Figure 4

Effect of fertilizers on lateral root diameter. (A-E) 2nd - 6th LRD respectively. (F) mean lateral root diameter (MLRD). Error bars represent 1SE of the mean (n = 9). † means significant differences among the six split-root treatments at P < 0.05. Significant differences are indicated by different lowercase letters at P < 0.05.

Figure 5

Effect of fertilizers on (A) SRL, (B) RSAB, (C) RTD, and (D) BI. SRL, specific root length; RSAB, ratio of root surface area to root dry biomass; RTD, root tissue density; BI, branch intensity. Error bars represent 1SE of the mean (n = 3). Significant differences are indicated by different lowercase letters at P < 0.05.

Figure 6

Anatomy of roots from pear trees grown in CF-CF, BIO-BIO, and CF-BIO split-root boxes. (A) Cork layer thickness, (B) xylem thickness, (C) phloem thickness, (D) stele:root diameter ratio (SDRD), (E) vessel density (VD), (F) stele radial, (G) vessel number in stele, (H) vessel diameter, and (I) cross-sectional area of the xylem (CSAX). LRIA: lateral root insertion angle. Error bars represent 1SE of the mean ($n = 9$). According to LSD tests, ns indicates non-significant difference among fertilized chambers at $P > 0.05$; significant differences are indicated by different lowercase letters at $P < 0.05$; † means significant differences among CF-CF and BIO-BIO conditions at $P < 0.05$.

Figure 7

Root nutrient content and activity (A) Carbon concentration, (B) N concentration, (C) C/N ratio, (D) and root activity. Error bars represent 1SE of the mean ($n = 3$). † means significant differences ($P < 0.05$) among the NF-NF, BIO-BIO and BIO-CF boxes at $P < 0.05$, § means significant differences among the twelve chambers of the boxes, and * means significant differences between the left and right chambers in the same box, according to LSD ($P < 0.05$) tests.

Figure 8

Pearson's correlations show the relationships between root system architecture and soil properties. Bars in shades of blue represent a higher correlation coefficient (r), compared to the control, bars in shades of red represent a lower correlation coefficient (r), compared to the control. Empty grids mean no difference. * P < 0.05, ** P < 0.01. SOM, soil organic matter; TN, total soil nitrogen; AP, available phosphorus; AK, available potassium; C/N, ratio of soil organic carbon to soil total nitrogen; TLRL, total lateral root length; TLRS, total lateral root surface; TLRV, total lateral root volume; TLRN, total lateral root number; MID, mean inter-branch density; RA, root activity; SRL, specific root length; RSAB, ratio of root surface area to root dry biomass; RTD, root tissue density; BI, branch intensity; MRD, mean lateral root diameter; RCC, root carbon concentration; RNC, root nitrogen concentration; RCC/RNC, ratio of root carbon concentration to root nitrogen concentration.

Figure 9

Linear regression analysis of the relationships between pear tree root traits and root nutrient content. The filled circles represent the fertilizer used: blue-NF, green-CF, and purple-BIO. TLRL, total lateral root length; TLRN, total lateral root number; SRL, specific root length; RTD, root tissue density; MRD, mean lateral root diameter; RCC, root carbon concentration; RNC, root nitrogen concentration; RCC/RNC, ratio of root carbon concentration to root nitrogen concentration. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 10

Redundancy analysis of twelve RSA traits, root nutrient content, and soil properties. SOM, soil organic matter; TN, total soil nitrogen; AP, available phosphorus; AK, available potassium; C/N, ratio of soil organic carbon to soil total nitrogen; TLRL, total lateral root length; TLRS, total lateral root surface; TLRV, total lateral root volume; TLRN, total lateral root number; MID, mean interbranch density; RA, root activity; SRL, specific root length; RSAB, ratio of root surface area to root dry biomass; RTD, root tissue density; BI, branch intensity; RCC, root carbon concentration; RNC, root nitrogen concentration; RCC/RNC, ratio of root carbon concentration to root nitrogen concentration.

Figure 11

Overview of the main RSA traits, root anatomical structure, and nutrient content in response to nutrient heterogeneity. Colored solid lines indicate primary roots and the different orders of lateral roots, line thickness indicates relative root diameter. The roots of a pear tree were placed in planting boxes such that half the roots grew in one chamber and half in a second chamber, these chambers were filled with soil and supplemented with bio-organic (right/grey square), or chemical fertilizer or unsupplemented (left/orange square). The gradually narrowing purple triangles, and their direction, represents the reduction of variables. RSA traits include global root parameters (such as total lateral root length, total lateral root number, total lateral root surface and volume), SRL and RTD. Root anatomical structures are the cork layer, xylem, phloem, and vessels. Root nutrition consisted of RCC and RNC. The growth of plants was evaluated by height and trunk girth. NF, no fertilizer; CF, chemical fertilizer; BIO, bio-organic fertilizer;

SRL, specific root length; RTD, root tissue density; RCC, root carbon concentration; RCN, root nitrogen concentration; LRs, lateral roots.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterials.docx](#)