

Strength and Size of Phosphorus-Rich Patches Determine the Foraging Strategy of *Neyraudia reynaudiana*

Liping Cai (✉ cailiping@fafu.edu.cn)

Fujian Agriculture and Forestry University <https://orcid.org/0000-0002-8216-197X>

Yuzhen Wang

Fujian Agriculture and Forestry University

Mulualem Tigabu

Swedish University of Agricultural Sciences

Xiaolong Hou

Fujian Agriculture and Forestry University

Pengfei Wu

Fujian Agriculture and Forestry University

Chuifan Zhou

Fujian Agriculture and Forestry University

Xiangqing Ma

Fujian Agriculture and Forestry University

Liping Cai

Fujian Agriculture and Forestry University

Research article

Keywords: Nutrient foraging, Nutrient patches, Root morphological plasticity, Root physiological plasticity, Phosphorus stress

Posted Date: August 27th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-54026/v1>

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

[Read Full License](#)

Version of Record: A version of this preprint was published at BMC Plant Biology on December 7th, 2020. See the published version at <https://doi.org/10.1186/s12870-020-02738-0>.

Abstract

Background: Under natural conditions, soil nutrients are heterogeneously distributed, and plants have developed adaptation strategies, such as root morphological plasticity and/or physiological plasticity, to efficiently forage patchily distributed nutrient. Most previous studies examined either patch strength or patch size separately and focused mainly on root morphological plasticity, thus the effects of both patch strength and size on morphological and physiological plasticity are not well understood. In this study, we examined the foraging strategy of *Neyraudia reynaudiana* (Kunth) Keng ex Hitchc, a pioneer grass colonizing degraded sites, with respect to patch strength and size in heterogeneously distributed phosphorus (P), and how foraging patchily distributed P affects total plant biomass. Plants were grown in sand-culture pots divided into $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{6}$ compartments and full size and supplied with 0 + 0/30, 0 + 7.5/30 and 7.5 + 0/30 mg P/kg dry soil as KH_2PO_4 or 0 + 15/15, 0 + 18.5/ 18.5, 7.5 + 15/15 mg kg^{-1} in the homogenous treatment. The first amount was the P concentration in the central region, and that the second amount was the P concentration in the outer parts of the pot.

Results: After 3 months of growth under experimental conditions, significantly ($p < 0.05$) high root elongation, root surface area, root volume and average root diameter was observed in large patches with high patch strength. Roots absorbed significantly more P in P-replete than P-deficient patches. Whole plant biomass was significantly higher in larger patches with high patch strength than small patches and homogeneous P distribution.

Conclusion: The result demonstrates that root morphological and physiological plasticity are important adaptive strategies for foraging patchily distributed P and the former is largely determined by patch strength and size. The results also establish that foraging patchily distributed P resulted in increased total plant biomass compared to homogeneous P distribution.

Introduction

Under natural conditions, soil nutrients are heterogeneously distributed and patches with different sizes, nutrient availability and nutrient content are mosaic in space [1]. This patchily distribution is a result of the spatio-temporal variation in litter inputs, decomposition and subsequent release of nutrients [2]. Evidence shows that spatially heterogeneous distribution of nutrients in soils occurs within ranges reachable by plant roots; for instance, within 1 m or shorter distance in sagebrush steppe [3] and beyond 2 m distance in a woodland soil [4]. To forage localized soil nutrient patches, plants have evolved adaptive mechanisms; notably deployment of more roots in nutrient-rich patches – the so called root morphological plasticity [5, 6]. Root morphology determines the spatial extent of soil occupied by root system and the size of root-soil contact surface, which directly affects the ability of the root to forage nutrients. Most plants forage nutrients in the heterogeneous space through proliferation of lateral roots, increased root biomass and root length and other morphological changes of the root system in nutrient-rich patches; thereby regulating the contact range and area of root-soil [7, 8]. Another mechanism by which plants forage nutrient in heterogeneous environment is through increasing nutrient uptake rates in

nutrient-rich patches – the so called physiological plasticity [6, 9]. In addition, heterogeneous nutrient supply may enhance growth and biomass production, depending on the species [10, 11].

Nutrient foraging in spatially heterogeneous environment is not only related to plant species, but also affected by nutrient patch attributes, nutrient elements and overall nutrient supply. Dunbabin et al. [12] observed significant differences in root proliferation in response to nitrate supply between *Lupinus angustifolius* and *Lupinus pilosus*. Drew [13] demonstrated that nitrogen (N) is relatively easy to diffuse in soil, and plants do not need root hyperplasia to acquire nitrogen in heterogeneous patches, while plants acquire the difficult-to-move phosphorus (P) by root hyperplasia. However, the experiment on *Agropyron desertorum* showed opposite results that the new roots are sensitive to N patches but insensitive to P patches [14]. The fine roots of *Picea sitchensis* seedlings were significantly proliferated in the NO₃⁻, NH₄⁺ or P nutrient patches [15]. These studies demonstrate that generalization of root responses to patchily distributed nutrients is still far away to make and the responses are species-specific. The efficacy of root plasticity is also mainly determined by the strength and size of the nutrient-replete patch [16, 17]. Patch strength is defined as the difference in P concentration between neighboring patches, with patches with high strength having a large P concentration compared with the adjacent patch. Most previous studies investigated root proliferation in response to patch strength; i.e. the concentration gradient between nutrient-rich and nutrient-poor patches [8]; however, few studies have examined root morphological responses to varying patch strength and patch size simultaneously [11, 17, 18]. Therefore, empirical evidence on how patch strength and patch size influence the root morphological and physiological plasticity, thereby enhancing nutrient foraging in heterogeneous nutrient environment, is still scarce.

In this study, we demonstrated the effects of patch strength and patch size on root morphological and physiological plasticity using *Neyraudia reynaudiana* (Kunth) Keng ex Hitchc as a model plant. *N. reynaudiana* is a fascinating perennial grass species that can grow in highly degraded and barren land, including rock outcrops, gravel heaps, excavated weakly differentiated parent material slopes, retaining wall stones, and in almost soilless kaolin mines, limestone mines, coal mines, gold and copper mines and tailings wastelands [19]. As a result, it is widely planted in East Asia for soil and water conservation purposes [20]. Previous studies focused on understanding the mechanisms by which this species cope up with low nutrient stress and thrives well on degraded sites. For instance, Feng et al. [21] found the endophytic diazotroph population with high nitrogenase activities in the stems of the *N. reynaudiana*, which allows accumulation of large amounts of N nutrition; Yang [22] demonstrated symbiotic relationship between roots of *N. reynaudiana* and VA mycorrhizal fungi; Feng et al. [23] also found symbiotic relation between roots of *N. reynaudiana* and phosphate-solubilizing fungi; Cai [24] found increased activities of POD, SOD and CAT enzymes and increased soluble protein content in leaves and acidic root exudates under low nutrient stress. Furthermore, Cai [24] and Hou et al. [8] observed changes in root morphological traits in response to low P supply. These studies had, however, been made under homogeneous low nutrient environment; except the study by Hou et al. [8], which demonstrated root morphological plasticity in response to patch strength only in heterogeneous P environment. Foraging strategy in response to patch mosaics with different sizes and strengths has not been studied in this

species. An understanding of the foraging strategy is a key to unravel the adaptation mechanisms of *N. reynaudiana* to extremely harsh environment and efficient use of the meager resources.

The main objective was to examine the effects of patch size and patch strength on root morphological traits, root P contents and physiological plasticity and whole-plant biomass production of *N. reynaudiana*. The study addressed the following research questions: (1) is root proliferation in P-rich patches influenced by patch size and patch strength?; i.e. is root morphological plasticity a major foraging strategy to patchily distributed P?; (2) does root P content vary with patch size and strength?, and does physiological plasticity play an adaptive role in foraging patchily distributed P? (3) is total plant biomass higher in heterogeneous than homogenous P distribution condition, and if so is it related to patch size and patch strength? We hypothesized that (1) *N. reynaudiana* deploys more roots in P-replete patches with higher patch strength and large patch size than in patches with low patch strength and small patch size; (2) root P content will be higher in roots grown in P-replete than P-deficient patches and varies with patch strength and patch size, thus physiological plasticity plays an important adaptive role (3) total plant biomass is expected to be higher in heterogeneous than homogeneous P distribution and varies with patch strength and patch size in heterogeneous distribution due to increased uptake of nutrients.

Results

Selective deployment of roots in spatially heterogeneous P distribution over time

There were statistically significant differences in the combined root morphological traits in response to patch strength, patch size, compartments (high P versus low P) and their interactions over time (Table 1). When the root morphological traits were considered separately, no significant effects of patch strength \times patch size on total root length and patch size \times compartment on root length and root volume were detected. Generally, all root morphological traits increased over time from 171 cm after 30 days of growth to 3285 cm after 90 days in total root length, from 16 cm² after 30 days to 391 cm² after 90 days in root surface area, from 0.3 mm after 30 days to 0.6 mm after 90 days in average root diameter and 0.12 cm³ after 30 days to 2.67 cm³ after 90 days in root volume. The total root length and average root diameter increased with decreasing patch strength while root surface area and root volume increased with increasing patch strength across all levels of patch size. Across all levels of patch strength, all root morphological traits increased with increasing patch size. Total root length after 30 days of growth was higher in large and medium patches with high P than low P when the patch strength was high with or without initial encounter with P-enriched patch (Fig. 1 top panel; T1–T2; T9–10), while moderate patch strength generally favored root elongation in large patches compared to medium and small patches (Fig. 1 top panel; T5–T8). Root elongation after 60 days of growth was still high in P-replete large patches while the opposite was observed in medium patches and no difference could be discerned in small patches when the patch strength was high (Fig. 1 middle panel; T1–T3). On the contrary, root

elongation was higher in large patches with low P than high P when the patch strength was moderate (Fig. 1 middle panel, T5), while it was higher in high P than low P small patches (Fig. 1 middle panel; T7). Plants grown initially in P-enriched patch produced longer roots when they were subsequently exposed to P-replete than P-deficient medium size patches (Fig. 1 middle panel; T10). After 90 days of growth, root elongation was still high in P-replete large patch when the patch strength was high, in P-replete medium patch when the patch strength was moderate and when plants grown initially in P-enriched patch followed by high patch strength (Fig. 1 bottom panel; T1, T6, T10).

Table 1
Results of multivariate ANOVA to examine the effects of patch strength and patch size on root morphological traits in low P and high P compartments.

Source	Wilks Lambda	d.f	F	<i>p</i>
Patch strength (S)	0.01	10	84.9	0.0001
Patch size (Ps)	0.02	15	28.8	0.0001
Compartment (C)	0.51	5	8.6	0.0001
Time (T)	0.01	8	168.5	0.0001
S × Ps	0.02	30	10.9	0.0001
S × C	0.44	10	4.5	0.0001
Ps × C	0.37	15	3.5	0.001
S × T	0.024	16	47.72	0.001
Ps × T	0.136	16	18.55	0.001
C × T	0.646	8	6.41	0.001
S × Ps × C	0.34	30	1.9	0.007
S × Ps × T	0.086	32	11.51	0.007
S × C × T	0.425	16	6.50	0.001
Ps × C × T	0.577	16	3.97	0.001
S × Ps × C × T	0.271	32	20.84	0.001

Large root surface area after 30 days of growth was observed in P-replete than P-deficient large and medium patches when the patch strength was high (Fig. 2 top panel; T1 and T2). However, root surface area didn't differ between P-replete and P-poor patches when the patch strength was moderate, but it was considerably higher in large patches than small patches, particularly in P-replete patches (Fig. 2 top panel; T5–T8). Plants initially grown in P-enriched patch produced larger root surface area in P-replete large and medium size patches (Fig. 2 top panel; T9 and T10). After 60 days of growth, root surface area was still

larger in P-replete than P-deficient large patch with high patch strength while the reverse was observed when the patch strength was moderate (Fig. 2 middle panel; T1 and T5). Under moderate patch strength, root surface area of plants was larger in P-poor than P-replete medium size patch but larger in P-replete than P-poor small patch (Fig. 2 middle panel; T6 and T7). There was no significant difference in root surface area in plants initially grown in P-enriched patch irrespective of the patch size (Fig. 2 middle panel; T9–T12). Larger root surface area was still observed after 90 days of growth in P-replete than P-poor medium size patches with high and moderate patch strength (Fig. 2 bottom panel; T2 and T6).

Root volume of plants after 30 days of growth was larger in small patch than in large and medium patches when the patch strength was high, but no significant difference was observed between P-replete and P-poor patches (Fig. 3 top panel; T1–T4). When the patch strength was moderate, root volume was larger in P-poor than P-replete medium size patch, while it was larger in P-replete than P-poor small size patch (Fig. 3 top panel; T6 and T7). Root volume was also larger in P-replete than P-deficient medium size patch in plants initially grown in P-enriched patch (Fig. 3 top panel; T10). After 60 days of growth, root volume was larger in P-replete than P-deficient large patch when the patch strength was high, while it was larger in P-poor large patch when the patch strength was moderate (Fig. 2 middle panel; T1 and T5), but no significant difference was observed in plants initially grown in P-enriched patch (Fig. 3 middle panel; T9–T12). Root volume after 90 days of growth was larger in P-replete than P-deficient large and medium size patches when the patch strength was high (Fig. 3 bottom panel; T1 and T2). When the patch strength was moderate, root volume was generally larger in large than small patches (Fig. 3 bottom panel; T5–T8) while no significant difference was observed among levels of patch size in plants initially grown in P-enriched patches (Fig. 3 bottom panel; T9–T12).

Average root diameter after 30 days of growth didn't differ significantly between patch irrespective of the patch size and the patch strength, but it was higher for the heterogeneous than homogeneous P distribution (Fig. 4 top panel). Plants grown for 90 days had bigger average root diameter when grown in P-replete than P-poor large patch with moderate patch strength (Fig. 4 middle panel; T5), while average root diameter of plants after 90 days of growth was higher in P-replete than P-deficient large and medium size patches with high patch strength (Fig. 4 bottom panel; T1 and T2), but it was lower in P-replete than P-poor large patch with moderate patch strength (Fig. 4 bottom panel; T5). All root morphological traits were similar in all patches in the homogenous P supply treatments.

Root dry mass

After 90 days of growth, root dry mass of plants grown in high patch strength (0 and 30 mg.kg⁻¹) was larger in large and medium size patches with high P than low P concentration whereas root dry mass didn't differ between high P and low P patches when plants were grown under moderate patch strength (7.5 and 30 mg kg⁻¹) and in initially P-enriched patch as well as homogenous P supply treatments (Table 2). As a whole, the morphological plasticity in root dry mass was higher under heterogeneous than homogeneous P-distribution, particularly in large and medium patches when the patch strength was high;

in medium patch when the patch strength was moderate; and in small patch when seedlings were grown in initially P-enriched patch (Table 2).

Table 2

Root dry mass and morphological plasticity (Mplast) after 90 days of growth in spatially heterogeneous and homogeneous P supply (mean \pm SE). For each level of patch strength, means followed by different lower and upper case letter (s) across columns are statistically different.

		Root Dry Mass (g)		
Patch strength	Patch size	Low P	High P	Mplast
0 + 0/30	Large	0.84 \pm 0.29b	1.11 \pm 0.18b	1.41 \pm 0.5B
	Medium	0.71 \pm 0.24ab	1.02 \pm 0.01b	1.58 \pm 0.6B
	Small	0.34 \pm 0.07a	0.39 \pm 0.04a	1.22 \pm 0.3A
	Homogeneous	0.36 \pm 0.07a	0.36 \pm 0.07a	1.00 \pm 0.0
0 + 7.5/30	Large	0.74 \pm 0.11b	0.70 \pm 0.19a	0.95 \pm 0.3A
	Medium	0.59 \pm 0.10ab	0.68 \pm 0.01a	1.16 \pm 0.2B
	Small	0.50 \pm 0.09a	0.57 \pm 0.15a	1.15 \pm 0.2AB
	Homogeneous	0.53 \pm 0.04ab	0.53 \pm 0.04a	1.00 \pm 0.0
7.5 + 0/30	Large	0.56 \pm 0.07b	0.51 \pm 0.16a	0.93 \pm 0.4A
	Medium	0.37 \pm 0.03a	0.41 \pm 0.03a	1.13 \pm 0.1A
	Small	0.25 \pm 0.09a	0.32 \pm 0.09a	1.60 \pm 1.1B
	Homogeneous	0.40 \pm 0.06a	0.40 \pm 0.06a	1.00 \pm 0.0

P contents, Translocation and P use efficiency

The root P content varied significantly ($P < 0.01$) between high P- and low P-patches irrespective of the patch strength and patch size, as well as for the first and second order interaction. In high patch strength, root P content was higher in large patches with high P concentration than in either medium or small patches (Table 3). Under moderate patch strength, the root P content didn't differ among different patch sizes. When the plant was initially grown in P-enriched patch, the root P content was higher in medium size patches with low P concentration than large and small patches. As a whole, the physiological plasticity (Pplast) was higher than 1 although not significantly different in response to patch strength and patch size (Table 3).

Table 3

Root P content (mg/g dry mass) and physiological plasticity (Pplast) after 90 days of growth in spatially heterogeneous and homogeneous P supply (mean \pm SE). For each level of patch strength, means followed by different lower and upper case letter (s) across columns are statistically different.

		Root P Content (mg)		
Patch Strength	Patch Size	High P-Patch	Low P-Patch	Pplast
0 + 0/30	Large	0.79 \pm 0.04b	0.67 \pm 0.17a	1.26 \pm 0.23A
	Medium	0.41 \pm 0.01a	0.37 \pm 0.08a	1.16 \pm 0.18A
	Small	0.38 \pm 0.03a	0.27 \pm 0.03a	1.46 \pm 0.22A
	Homogeneous	0.34 \pm 0.04a	0.34 \pm 0.05a	1.00 \pm 0.00A
0 + 7.5/30	Large	0.47 \pm 0.02a	0.47 \pm 0.03a	0.98 \pm 0.09A
	Medium	0.43 \pm 0.08a	0.37 \pm 0.03a	1.19 \pm 0.17A
	Small	0.43 \pm 0.07a	0.40 \pm 0.03a	1.07 \pm 0.11A
	Homogeneous	0.46 \pm 0.02a	0.46 \pm 0.02a	1.00 \pm 0.00A
7.5 + 0/30	Large	0.87 \pm 0.06a	0.84 \pm 0.06ab	1.05 \pm 0.14A
	Medium	0.94 \pm 0.01a	1.07 \pm 0.01c	0.87 \pm 0.05A
	Small	1.02 \pm 0.08a	0.98 \pm 0.08bc	1.05 \pm 0.13A
	Homogeneous	0.66 \pm 0.01a	0.66 \pm 0.01a	1.00 \pm 0.00A

The leaf P content varied significantly with respect to patch strength ($p < 0.01$), patch size ($p = 0.01$) and their interaction ($p = 0.004$). The leaf P content was lower in plants grown in initially P-enriched patch than in patches with high and moderate patch strength (Table 4). The leaf P content was higher in large and medium patches when the concentration gradient between patches was high than small patches and under homogeneous P supply. Similarly, stem P content varied significantly with respect to patch strength ($p < 0.001$), patch size ($p < 0.001$) and their interaction ($p < 0.001$). Stem P content was lower in plants grown under initially P-enriched patch than in both high and moderate patch strengths (Table 4). When the concentration gradient between patches was high, stem P was high in large patch than medium and small patches while it was higher in medium patch than high and small patches when the concentration gradient was moderate.

Table 4

P content (mg) P translocation (%) and use efficient of *Neyraudia reynaudiana* after 90 days of growth in spatially heterogeneous and homogeneous P supply (mean \pm SE). For each level of patch strength, means followed by different letter across within the column are statistically different.

Patch Strength	Patch Size	Leaf P (mg)	Stem P (mg)	P-Translocation (%)	P Use-Efficiency
0 + 0/30	Large	9.31 \pm 0.66b	4.95 \pm 0.03c	90.8 \pm 1.05a	0.92 \pm 0.04b
	Medium	8.69 \pm 0.71b	2.99 \pm 0.15b	93.6 \pm 1.19a	1.00 \pm 0.05b
	Small	6.03 \pm 0.11a	1.41 \pm 0.08a	91.9 \pm 0.16a	0.76 \pm 0.01a
	Homog.	6.23 \pm 0.05a	2.79 \pm 0.29b	93.1 \pm 0.67a	0.87 \pm 0.01ab
0 + 7.5/30	Large	11.09 \pm 0.31a	3.26 \pm 0.28a	93.8 \pm 0.59ab	1.01 \pm 0.07a
	Medium	10.58 \pm 1.16a	7.28 \pm 0.87b	95.7 \pm 0.08b	0.76 \pm 0.05a
	Small	11.52 \pm 0.27a	3.74 \pm 0.31a	94.8 \pm 0.35ab	0.78 \pm 0.04a
	Homog.	9.06 \pm 0.68a	3.10 \pm 0.71a	92.9 \pm 0.61a	0.87 \pm 0.09a
7.5 + 0/30	Large	0.48 \pm 0.06b	0.56 \pm 0.04b	10.6 \pm 0.32b	0.82 \pm 0.06a
	Medium	0.39 \pm 0.02ab	0.37 \pm 0.02a	9.6 \pm 0.24ab	1.30 \pm 0.10b
	Small	0.25 \pm 0.07a	0.25 \pm 0.05a	9.4 \pm 0.41ab	1.76 \pm 0.13c
	Homog.	0.26 \pm 0.02a	0.40 \pm 0.04a	8.8 \pm 0.11a	0.84 \pm 0.08a

The P translocation to the shoots and P use efficiency also varied significantly with respect to patch strength ($p < 0.001$), patch size ($p < 0.05$) and their interaction ($p < 0.001$). Translocation of P to the shoots was much lower in plants grown under initial P-enriched patch followed by high patch strength than those grown in moderate patch strength (Table 4). However, the P use efficiency was higher when the plant grew in initially P-enriched patch followed by high concentration gradient in small patches than large and medium patches (Table 4).

Biomass production and Sensitivity index

Total root dry mass, shoot dry mass, total plant dry mass and root to shoot dry mass ratio varied significantly ($p < 0.05$) with respect to patch strength, patch size and interaction between patch strength and patch size. Total root dry mass after 90 days of growth was significantly higher in large patch than small patch and homogeneous P distribution across all concentration gradient between patches, except moderate patch strength (Table 5). Shoot dry mass was higher in large and medium patch than small patch and homogenous P supply for seedlings grown under conditions of high (0 and 30 mg.kg⁻¹) and moderate (7.5 and 30 mg.kg⁻¹) P concentration gradient between patches. For plants initially grown in P-

enriched patch (7.5 + 0 and 30 mg.kg⁻¹), shoot dry mass was higher in large than medium and small patches, but statistically similar with those grown under homogenous P supply. The total plant dry mass after 90 days of growth was higher in large patches than small patches (Table 6). The root to shoot dry mass ratio was larger in large and medium patches than small patch and homogenous P distribution (Table 6) when the concentration gradient was high (0 and 30 mg.kg⁻¹). As a whole, whole-plant biomass was sensitive to the spatial distribution of P, as evidenced from statistically significant difference ($p < 0.05$) in sensitivity index across different P concentration gradients and patch size (Table 6). Plants grown under high concentration gradient between patches produced more biomass than those grown under homogenous P supply, particularly in large and medium patches.

Table 5

Total root and shoot dry mass (g) after 30, 60 and 90 days of growth of *N. reynaudiana* seedlings in spatially heterogeneous and homogeneous P supply (mean \pm SE). For each level of patch strength, means followed by different letter across columns are statistically different.

Patch attributes		Dry mass, 30 days		Dry mass, 60 days		Dry mass, 90 days	
Patch Strength	Patch Size	Root	Shoot	Root	Shoot	Root	Shoot
0 + 0/30	Large	0.02 \pm 0.002a	0.36 \pm 0.09b	0.9 \pm 0.1b	4.7 \pm 0.1b	2.0 \pm 0.4b	13.0 \pm 0.2d
	Medium	0.01 \pm 0.003a	0.20 \pm 0.02a	0.6 \pm 0.1a	3.8 \pm 0.3a	1.7 \pm 0.2b	11.7 \pm 0.2c
	Small	0.01 \pm 0.006a	0.31 \pm 0.02b	0.5 \pm 0.1a	3.7 \pm 0.2a	0.7 \pm 0.03a	5.6 \pm 0.1a
	Homog.	0.01 \pm 0.002a	0.32 \pm 0.03b	0.6 \pm 0.03a	3.8 \pm 0.5a	0.7 \pm 0.1a	7.9 \pm 0.5b
0 + 7.5/30	Large	0.03 \pm 0.005b	0.39 \pm 0.02c	0.5 \pm 0.05b	4.4 \pm 0.2c	1.4 \pm 0.2a	14.5 \pm 1.0c
	Medium	0.02 \pm 0.001ab	0.28 \pm 0.02b	0.3 \pm 0.01a	2.8 \pm 0.7a	1.3 \pm 0.1a	13.4 \pm 0.4c
	Small	0.02 \pm 0.003ab	0.25 \pm 0.03b	0.2 \pm 0.05a	2.3 \pm 0.6a	1.1 \pm 0.2a	11.9 \pm 0.2b
	Homog.	0.01 \pm 0.001a	0.16 \pm 0.03a	0.3 \pm 0.06a	3.7 \pm 0.9b	1.1 \pm 0.1a	10.3 \pm 0.4a
7.5 + 0/30	Large	0.03 \pm 0.005b	0.25 \pm 0.04a	0.4 \pm 0.03a	4.0 \pm 0.5b	1.1 \pm 0.1c	10.1 \pm 0.6b
	Medium	0.02 \pm 0.007a	0.22 \pm 0.05a	0.4 \pm 0.02a	3.9 \pm 0.1ab	0.8 \pm 0.1b	8.1 \pm 0.3b
	Small	0.03 \pm 0.006b	0.38 \pm 0.01b	0.4 \pm 0.04a	3.6 \pm 0.9a	0.6 \pm 0.01a	6.1 \pm 0.5a
	Homog.	0.02 \pm 0.001a	0.24 \pm 0.23a	0.4 \pm 0.04a	5.1 \pm 0.5c	0.8 \pm 0.1b	9.0 \pm 1.5b

Table 6

Root dry mass to shoot dry mass ratio and total dry mass together with sensitivity index after 90 days of growth of *N. reynaudiana* in spatially heterogeneous and homogeneous P supply (mean \pm SE). For each level of patch strength, means followed by different letter across columns are statistically different.

Patch Strength	Patch Size	Root:Shoot Ratio	Total Dry Mass (g)	Sensitivity Index
0 + 0/30	Large	0.15 \pm 0.03b	14.99 \pm 0.45d	1.75 \pm 0.08b
	Medium	0.15 \pm 0.02b	13.45 \pm 0.21c	1.57 \pm 0.13b
	Small	0.13 \pm 0.01ab	6.37 \pm 0.11a	0.74 \pm 0.04a
	Homog.	0.09 \pm 0.01a	8.59 \pm 0.66b	
0 + 7.5/30	Large	0.10 \pm 0.01a	15.94 \pm 1.19b	1.40 \pm 0.15b
	Medium	0.09 \pm 0.004a	14.72 \pm 0.52b	1.30 \pm 0.08ab
	Small	0.09 \pm 0.02a	12.95 \pm 0.46a	1.14 \pm 0.06a
	Homog.	0.10 \pm 0.01a	11.37 \pm 0.47a	
7.5 + 0/30	Large	0.11 \pm 0.01b	11.18 \pm 0.63c	1.16 \pm 0.22b
	Medium	0.10 \pm 0.004ab	8.87 \pm 0.36b	0.92 \pm 0.18ab
	Small	0.09 \pm 0.01ab	6.64 \pm 0.49a	0.68 \pm 0.10a
	Homog.	0.09 \pm 0.001a	9.84 \pm 1.61bc	

Discussion

The results confirm our first hypothesis that selective deployment of roots in high P patches than low P patches; thus root morphological plasticity plays an adaptive role in foraging heterogeneous P distribution by this species. The observed root morphological response is significantly modulated by patch strength and size; i.e. the greater the patch strength and the larger the patch size, the greater the deployment of root in high P than low P patches would be. The significantly higher root morphological traits on the high P side in the split-P treatment imply that P-deficiency signal from the low P side may stimulate the growth of the roots located in the high-P zone. This rooting characteristics increase the chance of encountering nutrient-rich patches, thereby enabling plants to efficiently forage in a heterogeneous soil profile [25]. The increase in root morphological traits over time with slight differences among levels of patch strength and patch size is mainly ontogenic difference but suggests that the plant adjusts its root system to meet the P demand as growth advances.

Spatial heterogeneity of nutrient in soils is reflected in the scale of the distribution. During the growth and development process, plant roots experience nutrient patches of different scales, and plant roots make corresponding morphological response [26]. Studies have shown that plant roots have a threshold for the

“perception” of nutrient heterogeneity scale [27]. Under the condition of small heterogeneity scale, plants ignore the heterogeneity and regard it as homogeneous, and roots do not make plastic changes, or plasticity is very small. However, when the heterogeneity scale reached a certain threshold value, the root system cannot ignore the differences between heterogeneous environments, and a series of responses is triggered [27]. It has been shown that the difference in total root length in large, medium and small patches is not significant under the condition of high P, while the total root length in large patches can reach 3 times that of medium and small patches, and local root proliferation disappeared in small and medium patches under the condition of low phosphorus [1, 28–30]. Rhizomorphous clonal plants obtained nutrient resources by branching outwards, and the clonal plants were densely distributed in large patches with high nutrient content, while those in small patches were dispersed [31, 32]. This is in line with our findings that the root length, root surface area, root volume, and average root diameter in large patches were significantly higher than those in small patches.

In addition, the increased placement of roots in large patches with higher P concentration gradient could be related to the carbon cost for increased production of roots in smaller patches with low concentration gradient. Consequently, the plant has to make a “decision” on resource allocation to produce more roots in large patches to optimize nutrient capture, particularly for less mobile nutrients such as P. Interestingly all root morphological traits were low when the plant was initially grown in P-enriched patches than without initially encountering P (T9–T11 in Figs. 1–4). This indicates that *N. reynaudiana* is content with small amount of initial P availability, but slowly proliferated its root to forage patchily distributed P. As a whole, our results are consistent with previous studies that have demonstrated increased root deployment in localized nutrient-rich patches for a range of other species [5, 7, 8, 11, 17, 33, 34, 35].

The results also confirmed our second hypothesis that increased root P contents in high P- than low P- patch; suggesting that physiological plasticity plays an adaptive role in foraging patchily distributed nutrients in the soil by this species. Furthermore, the increased uptake of P in localized P-rich patches indicates that the species is highly efficient in resource acquisition. The fact that roots in patches with no P availability (0/30) had a certain amount of root P suggests internal redistribution of P to maintain P homeostasis. Similar results have been observed in low P tolerant Chinese fir genotypes [36]. While the translocation of P to the shoots and P content of leaf and stem were lower in plants initially grown in P-enriched patch followed by high P concentration gradient between patches, the P use efficiency was higher, especially in small patches. This indicates that this species has high P utilization efficiency when the availability of P is low. Utilization efficiency, defined as the amount of biomass per unit of nutrient present in the biomass, involves mechanisms such as remobilization of internal P, increased activity of enzymes that replace P in structural compounds or during metabolism [37, 38], or reduced consumption of P [39].

Total plant biomass is higher in heterogeneous than in homogeneous nutrient environment, but vary with patch strength and patch size, which confirms our third hypothesis. The total plant dry mass was significantly higher in large and medium patches in both high and low P concentration gradient between patches whereas plants initially grown in P-enriched patches produced more dry mass in large than small

patch and homogeneous P distribution. This is in line with the general notion that foraging patchily distributed nutrients results in increased whole-plant productivity and growth rates compared with homogeneous environment [10, 11, 33]. Generally, *N. reynaudiana* produced less root than shoots (i.e. low root to shoot ratio) across all treatments; suggesting that the species has high P utilization efficiency, as also observed for other species [40]. Shoot biomass production was favored by availability of high concentration of P in the growing media. This is indeed expected as P is the main growth-limiting nutrients in the study area while it is essential for various plant metabolic processes. Thus, biomass production is more sensitive to heterogeneous than homogeneous P supply.

Conclusions

The findings demonstrate that foraging strategy for patchily distributed nutrients in soils is highly dependent on both patch strength and patch size. *N. reynaudiana* efficiently forages patchily distributed P through deployment of more roots in P-replete patches than P-poor patches, and larger difference in patch strength and patch size induces deployment of more root in localized P-replete patches. The increased deployment of root in P-replete patches resulted in increased P content in leaves and stem, suggesting that physiological plasticity plays an adaptive role in foraging patchily distributed P, depending on patch size and strength. Thus, both root morphological plasticity and physiological plasticity are the main adaptation mechanisms to forage patchily distributed P by this species. The benefit of foraging heterogeneously distributed P is revealed in increased total plant biomass production in heterogeneous environment; thus biomass production is more sensitive to heterogeneous than homogeneous P distribution.

Materials And Methods

Experimental material

N. reynaudiana seedlings were used as experimental material to investigate their foraging strategy to spatially heterogeneous P distribution. Seeds of *N. reynaudiana* were purchased from a company in Kunming, Yunnan Province and sown in climate chamber set at 25 °C, 75% relative humidity, and a photoperiod of 12 h light (photon flux density 4000 lx) and 12 h dark. After 2 weeks, the germinants were transferred to nutrient rich humus substrate and left to grow in a greenhouse for four weeks. Seedlings with relatively uniform size (12 cm ± 1 cm in height, 4 cm ± 1 cm in root length, and 10 mg ± 1.5 mg in fresh weight) were selected for this experiment.

Experimental design and Growth conditions

To investigate the foraging strategy of *N. reynaudiana* seedlings to spatially heterogeneous P supply, a factorial experiment, which involved three levels of patch strength (0 + 0/30, 0 + 7.5/30 and 7.5 + 0/30 mg P. kg⁻¹) and three levels of patch size (small, medium and large) were established (Fig. 5). The treatment involved 7.5 + 0/30 mg P. kg⁻¹ was meant to examine whether initial encounter with P influences

subsequent root deployment in patches. In addition, a homogeneous P supply with three levels (15/15, 18.75/18.75 and 15/15 + 7.5 mg P·kg⁻¹ per patch), corresponding to the total P concentration in heterogeneous P supply, was included to compare total biomass production between heterogeneous and homogeneous P supply. The P concentrations used in this study were determined based on the soil conditions in southern China. A total of 12 treatments were applied in the experiment: 9 heterogeneous and 3 homogenous P treatments.

The experiment was conducted using pots (diameter: 40 cm, height: 32 cm), divided into ½, ¼ and 1/6 baffle less compartments using plastic separator to simulate large, medium and small patches, respectively. Each pot compartment was filled with a mixture of sand and sodium polyacrylate prior to application of different P concentrations as the sodium polyacrylate (0.5–1 mm in diameter) strongly adsorbed the applied P and hence prevented its movement between compartments but capable of releasing nutrients slowly and evenly and could be freely penetrated by roots. According to the weight of each pot, the sodium polyacrylate and washed river sand, the different concentrations of KH₂PO₄ solution (as P source) were mixed and packed in a volume ratio of 1:3 in each pot. In each treatment pot, one *N. reynaudiana* seedling was planted in a cylindrical tube (30 cm in length and 8 cm in diameter) filled with sand or sand mixed with 7.5 mg KH₂PO₄·kg⁻¹ at the center of the pot. Once the plants were placed correctly at the center of the treatment pots, the cylindrical tube and patch separators were removed carefully (Fig. 5). Each treatment had three replicates of independent plants.

The experiment was carried out in the greenhouse of the Forestry College, Fujian Agriculture and Forestry University under the following environmental conditions: 29.3 °C/23 °C (day/night); photon flux density of 21 mol quanta m⁻² d⁻¹, and ca. 42.7% and 67.7% relative humidity during the light and dark periods of the experiment, respectively. Seedlings were left to grow under this condition for three months. To meet the growth requirements for other nutrients, 50 mL of nutrient solution adjusted to a pH of 5.5 was supplied to each pot compartment every 5 days. Macro-nutrients were supplied according to a modified Hoagland solution as 0.51 gL⁻¹ KNO₃, 0.82 gL⁻¹ Ca(NO₃)₂·0.49 gL⁻¹ MgSO₄·7H₂O and 0.136 gL⁻¹ KCl (Chen et al. 1992). Micro-nutrients were also supplied according to Amon formula as 2.86 gL⁻¹ H₃B₃O₃, 0.08 gL⁻¹ CuSO₄·5H₂O, 0.22 gL⁻¹ ZnSO₄·7H₂O, 1.81 gL⁻¹ MnCl₂·4H₂O, 0.09 gL⁻¹ H₂MoO₄·H₂O and 20 gL⁻¹ Fe₂EDTA. The seedlings were watered every two to three days depending on the moisture content of the substrate.

Data collection and Statistical analysis

To examine temporal variation in root morphological traits, plants were harvested after one, two and three months of treatment application by first draining down the sand from each pot with water, and the roots were tied up in bundle from each compartment in both homogeneous and heterogeneous P treatments and thereafter carefully pulled out each seedling. All fine roots were collected from the growing media in each compartment. The roots from the different compartments were cleaned with distilled water separately, quickly dried with paper towels, and scanned with digital scanner (STD1600 Epson USA) with non-overlapping tiles. The root morphological traits (total root length, total surface area, total root volume

and average root diameter) from each compartment were determined by WinRhizo root analysis system (Version 4.0 B; Regent Instruments Inc, Canada). To determine dry matter of roots, shoots (stems and leaves) and whole-plant, the shoots and roots of the harvested plants were oven-dried first at 105 °C for 30 min and then at 79 °C until constant mass.

To examine the foraging strategy of *N. reynaudiana* seedlings to spatially heterogeneous P supply, four-way between-groups multivariate analysis of variance (MANOVA) was performed using time, patch strength, patch size and high P versus low P compartments as fixed independent variables while taking root morphological traits as dependent variables. The Bonferonni adjustment for multiple comparisons was employed to control the inflation of Type 1 error (Quinn and Keough, 2002). With four treatments in our study, time, patch strength, patch size and high versus low P compartments, we have six possible pair-wise comparisons, thus the Bonferonni adjusted p value was 0.0083 (0.05/6). Results of the statistical analyses were considered significant if $p < 0.0083$ and to show tendencies if $0.0083 < p < 0.05$. Root dry mass, P contents of roots, leaf and stem, percent P translocation to the shoot and P use efficiency were calculated for each treatment. P content was computed by multiplying P concentration of the sample by dry mass of the respective organ; P translocation was computed as a ratio of shoot P to total P; and P use efficiency was calculated the ratio of shoot dry mass to shoot P. Three-Way ANOVA was performed to examine differences in root dry mass and root P contents with respect to patch strength, patch size and between P-replete and P-deficient patches while Two-ANOVA was performed to determine the effects of patch strength and patch size on leaf and stem P contents, P translocation and P use efficiency.

To examine the foraging behavior and its benefit in whole-plant biomass production in response to patchily distributed P, root morphological plasticity (Mplast), root physiological plasticity (Pplast) and sensitivity index (SI) were calculated for each level of patch strength and patch size. Mplast for the heterogeneous treatment was computed as a ratio of root dry mass in high P-patch to that in low P-patch while for the homogeneous treatment it was computed as the ratio of root dry mass in two opposite patches. Pplast was computed as a ratio of root P content in high P-patch to that in low P-patch. Mplast and Pplast are expected to be one in the homogenous treatment, but greater than one in the heterogeneous treatment. SI was computed as the ratio of plant total biomass (total root dry mass and shoot dry mass) in the heterogeneous treatment to that in the homogenous treatment for each level of patch strength and patch size. Two-way ANOVA was performed to examine the effects of patch strength and patch size on shoot (leaf + stem) and total biomass (shoot + root) dry mass, Mplast, Pplast and SI. Means that showed significant differences were compared by Tukey's Post hoc test ($p < 0.05$). All statistical analyses were computed using SPSS Statistical Package (SPSS 20.0, SPSS Ins., Chicago, IL, U.S.A.).

Abbreviations

Phosphorus: P

Nitrogen: N

Root morphological plasticity: Mplast

Root physiological plasticity: Pplast

Sensitivity index: SI

Ethics approval and consent to participate:

Not applicable

Consent for publication:

Not applicable

Availability of data and materials:

Data will be made available up on request.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Data will be made available up on request.

Competing interests: The authors declare that they have no competing interests

Funding: This work was supported by the National Natural Science Foundation of China (grant number 2014BAD15B02 to Ma), and Innovation Science Foundation of Fujian Agriculture and Forestry University (grant number CXZX2016065 and CXZX2017090 to Cai).

Author Contributions: Conceptualization, L.C., M.T., X.M., X.H, P.W.; methodology, L.C., X.H., C.Z.; validation, M.T.; formal analysis, Y.W., L.C.; investigation, Y.W., L.C.; data curation, L.C.; writing—original draft preparation, L.C., Y.W. writing—review and editing, M.T.; visualization, L.C.; supervision, X.M.; project administration, X.M.; funding acquisition, X.M., L.C.

Acknowledgments: We thank the member of Institute of Forestry and Environment, Fujian Agriculture and Forestry University for lively discussions that improved the work. Huang Pengping, Hua Cong, Zhang Qiong are acknowledge for their laboratory assistance.

References

1. Liu YJ, Bortier MF, De Boeck HJ, Nijs I. Root distribution responses to three-dimensional soil heterogeneity in experimental mesocosms. *Plant Soil*. 2017;421:353–66.
2. McDowell RW, Hill SJ. Speciation and distribution of organic phosphorus in river sediments: A national survey. *Journal of Soil Sediment*. 2015;15:2369–79.
3. Jackson RB, Caldwell MM. The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. *Ecology*. 1993;74:612–4.
4. Farley RA, Fitter AH. Temporal and spatial variation in soil resources in deciduous woodland. *J Ecol*. 1999;87:688–96.
5. Wang L, Mou P, Jones RH. Nutrient foraging via physiological and morphological plasticity in three plant species. *Can J For Res*. 2006;36:164–73.
6. Nie YP, Chen HS, Wang KL, Ding YL. Rooting characteristics of two widely distributed woody plant species growing in different karst habitats of southwest China. *Plant Ecol*. 2014;215:1099–109.
7. He Y, Liao H, Yan X. Localized supply of phosphorus induces root morphological and architectural changes of rice in split and stratified soil cultures. *Plant Soil*. 2003;248:247–56.
8. Hou X, Tigabu M, Zhang Y, Ma X, Cai L, Wu P, Liu AQ, Wang C, Qiu H. Root plasticity, whole plant biomass, and nutrient accumulation of *Neyraudia reynaudiana* in response to heterogeneous phosphorus supply. *Journal of Soil and Sediment* 2017 17, 172–180.
9. Karthikeyan AS, Jain A, Nagarajan VK, Sinilal B, Sahi SV, Raghothama KG. *Arabidopsis thaliana* mutant *lpsi* reveals impairment in the root responses to local phosphate availability. *Plant Physiology and Biochemistry* 2014 77, 60–72.
10. Hutchings MJ, Wijesinghe DK. Performance of a clonal species in patchy environments: Effects of environmental contention yield at local and whole-plant scales. *Evol Ecol*. 2008;22:313–24.
11. Zhang Y, Zhou Z, Ma X, Jin G. Foraging ability and growth performance of four subtropical tree species in response to heterogeneous nutrient environments. *Journal of Forest Research*. 2010;15:91–8.
12. Dunbabin V, Rengel Z, Diggle A. *Lupinus angustifolius* has a plastic uptake response to heterogeneously supplied nitrate while *Lupinus pilosus* does not. *Aust J Agric Res*. 2001;52:505–12.
13. Drew MC. Comparison of the effect of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytologists*. 1975;75:479–90.
14. Jackson RB, Pockman WT, Hoffmann WA. The structure and function of root systems. In: Pugnaire FI, Valladares F. *Hand book of functional plant ecology*. New York: Eds.; Marcel Decker Inc; 1999. pp. 195–220.
15. Proe MF, Millard P. Effect of P supply upon seasonal growth and internal cycling of P in Sitka spruce *Picea sitchensis* (Bong.) Carr. seedlings. *Plant and Soil* 1995, 168–169, 313–317.
16. Hodge A. The plastic plant: Root responses to heterogeneous supplies of nutrients. *New Phytologists*. 2004;162:9–24.

17. Kume T, Sekiya N, Yano K. Heterogeneity in spatial P-distribution and foraging capability by *Zea mays*: Effects of patch size and barriers to restrict root proliferation within a patch. *Ann Bot.* 2006;98:1271–7.
18. Mou P, Jones RH, Tan Z, Bao Z, Chen H. Morphological and physiological plasticity of plant roots when nutrients are both spatially and temporally heterogeneous. *Plant Soil.* 2013;364:373–84.
19. Wang YZ, Cai LP, Zhou CF, Hou XL, Zou XH. Review of stress resistance and application of the pioneer plant *Neyraudia reynaudiana*. *Pratac Science.* 2017;34:1601–10. (in Chinese).
20. Cai LP, Wu PF, Hou XL, Ma XQ, Jiang S, Ren JJ. Effects of phosphorus stress on photosynthetic characteristics of pioneer plant *Neyraudia reynaudiana* on soil and water conservation. *J Soil Water Conserv.* 2012;26:281–5. (in Chinese).
21. Feng H, Guo YB, Lin RQ, Peng GX, Tan ZY. Preliminary studies on endophytic diazotrophs from *Neyraudia reynaudiana* and *Cynodon dactylon*. *Journal of Tropical Subtropical Botany.* 2009;17:465–70. (in Chinese).
22. Yang ST. Analysis on the relationship between the growth of the *Neyraudia reynaudiana* and the VA mycorrhiza. *Subtropical Soil Water Conservation.* 2008;20:16–8. 29. (in Chinese).
23. Feng H, Li YT, Zhang ZH, Wei XH, Guo YB. Screening, identification, and capability assessment of a phosphorus solubilizing fungus in rhizosphere of burma reed. *Microbiology China.* 2010;37:677–81. (in Chinese).
24. Cai LP. The mechanism of eco-physiological response to environmental stress for pioneer plant *Neyraudia reynaudiana* in collapsing hill area. PhD Dissertation, Fujian Agriculture and Forestry University, Fuzhou, China, 2012 (in Chinese).
25. Wijesing DK, John EA, Beurskens S, Hutchings MJ. Root system size and precision in nutrient foraging: Responses to spatial pattern of nutrient supply in six herbaceous species. *J Ecol.* 2001;89:972–83.
26. Wei HX, Guo P, Zheng HF, He XY, Wang PJ, Ren ZB, Zhai C. Micro-scale heterogeneity in urban forest soils affects? ne root foraging by ornamental seedlings of Buddhist pine and Northeast yew. *Urban Forestry and Urban Greening* 2017 28, 63–72.
27. Henke M, Sarlikioti V, Kurth W, Buck-Sorlin GH, Pages L. Exploring root developmental plasticity to nitrogen with a three-dimensional architectural model. *Plant Soil.* 2014;385:49–62.
28. Yano K, Kume T. Root morphological plasticity for heterogeneous phosphorus supply in *Zea mays* L. *Plant Production Science.* 2006;8:427–32.
29. Gao Y, Xing F, Jin YJ, Nie DD, Wang Y. Foraging responses of clonal plants to multi-patch environmental heterogeneity: Spatial preference and temporal reversibility. *Plant Soil.* 2012;359:137–47.
30. McNickle GG, Brown JS. When Michaelis and Menten met Holling: Towards a mechanistic theory of plant nutrient foraging behavior. *AOB Plants.* 2014. doi:10.1093/aobpla/plu066.
31. Peng YK, Luo FL, Li HL, Yu FH. Growth responses of a rhizomatous herb *Bolboschoenus planiculmis* to scale and contrast of soil nutrient heterogeneity. *Chinese Journal of Plant Ecology.* 2013;37:35–

43. (in Chinese).
32. Qian YQ, Luo D, Gong G, Han L, Ju GS, Sun ZY. Effects of spatial scale of soil heterogeneity on the growth of a clonal plant producing both spreading and clumping ramets. *J Plant Growth Regul.* 2014;33:214–21.
33. Nakamura R, Kachi N, Suzuki JI. Root growth and plant biomass in *Lolium perenne* exploring a nutrient-rich patch in soil. *J Plant Res.* 2008;121:547–57.
34. Mommer L, Visser EJW, Ruijven J, Caluwe H, Pierik R, Kroon H. Contrasting root behavior in two grass species: A test of functionality in dynamic heterogeneous conditions. *Plant Soil.* 2011;344:347–60.
35. Zhou C, Jiang W, Li Y, Hou X, Liu AQ, Cai L. Morphological plasticity and phosphorus uptake mechanisms of hybrid *Eucalyptus* roots under spatially heterogeneous phosphorus stress. *Journal of Forestry Research.* 2017;28:713–24.
36. Farooq TH, Tigabu M, Ma XQ, Zou XH, Liu AQ, Odén PC, Wu P. Nutrient uptake, allocation and biochemical changes in two Chinese fir cuttings under heterogeneous phosphorus supply. *iForest Biogeosciences Forestry.* 2018;11:411–7.
37. Li M, Welti R, Wang X. Quantitative profiling of *Arabidopsis* polar glycerolipids in response to phosphorus starvation. Roles of phospholipases Dz1 and Dz2 in phosphatidylcholine hydrolysis and digalactosyldiacylglycerol accumulation in phosphorus-starved plants. *Plant Physiol.* 2006;142(2):750–61.
38. Hammond JP, White PJ. Sucrose transport in the phloem: Integrating root responses to phosphorus starvation. *J Exp Bot.* 2008;59(1):93–109.
39. Shenoy VV, Kalagudi GM. Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnol Adv.* 2005;23(7–8):501–13.
40. Wu P, Tigabu M, Ma XQ, Odén PC, He Y, Yu X, He Z. Variations in biomass, nutrient contents and nutrient use efficiency among Chinese fir provenances. *Silvae Genetica.* 2011;60:95–105.

Figures

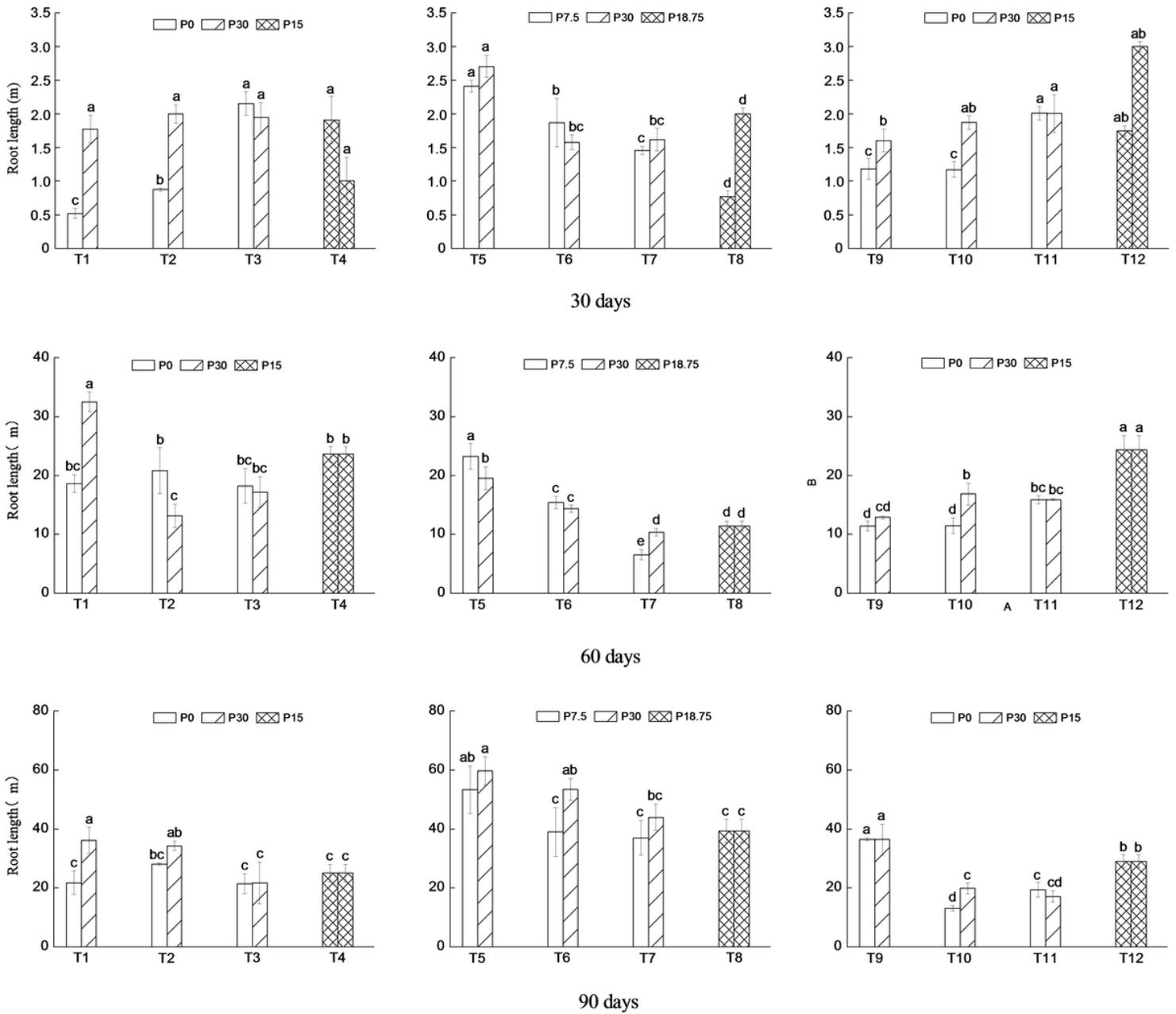


Figure 1

Total root length (m) of *N. reynaudiana* grown in high and low P patches under heterogeneous and homogenous P distribution for 30 (top panel), 60 (middle panel) and 90 days (bottom panel) where T1, T2 and T3 had high P concentration gradient between patches (0 and 30 mg.kg⁻¹), T5, T6 and T7 had moderate P concentration gradient (7.5 and 30 mg kg⁻¹), T9, T10 and T11 had initial P concentration of 7.5 mg kg⁻¹; i.e. P-enriched patch, and subsequently high P concentration gradient between patches (0 and 30 mg kg⁻¹); and T4, T8 and T12 had homogenous P distribution of 15, 18.5 and 7.5 + 15 mg kg⁻¹, respectively. Values are mean \pm SE (n = 3), * indicates significant difference at p \leq 0.05 and lowercase letters indicate significant difference between treatments.

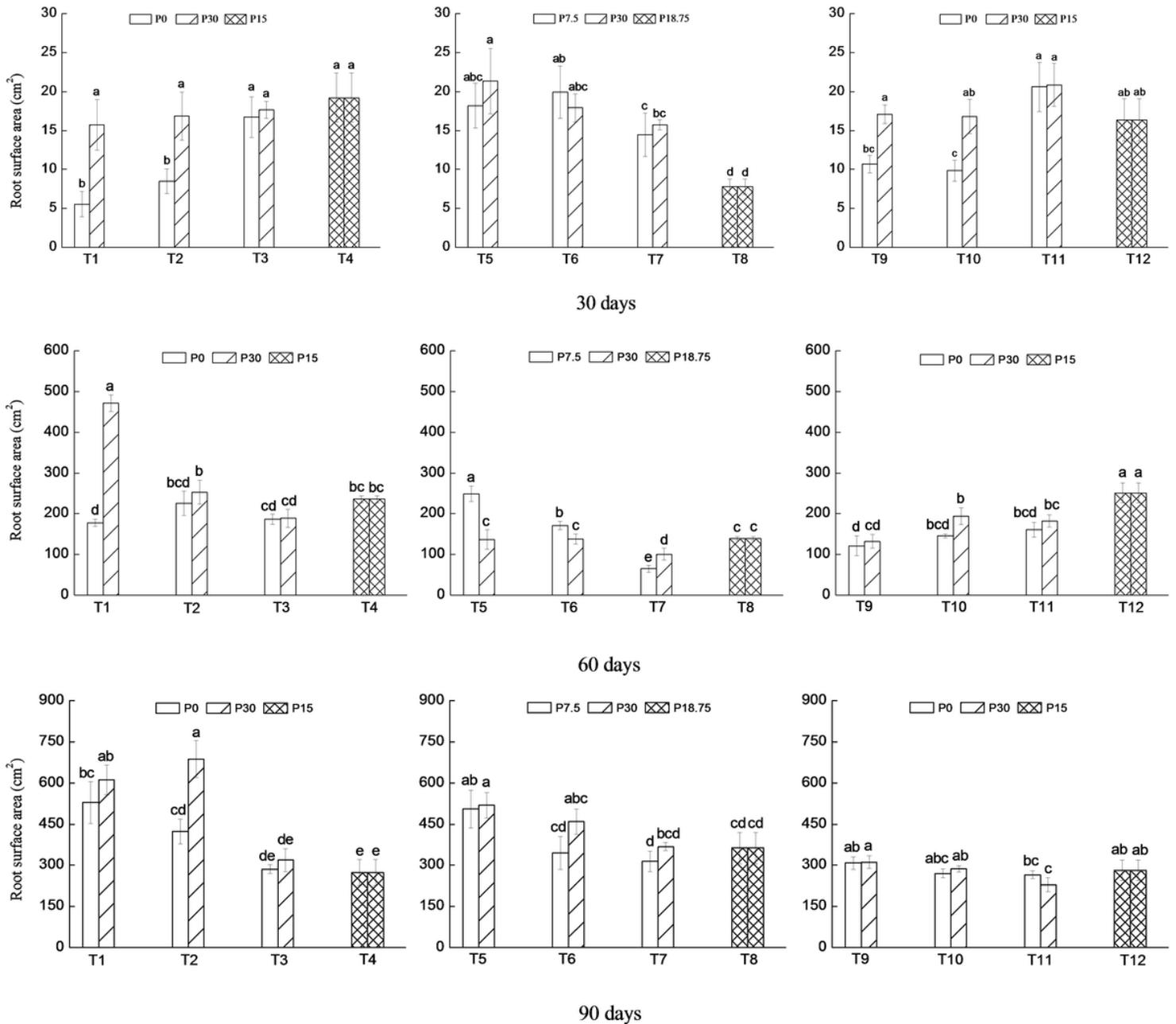


Figure 2

Root surface area (cm²) of *N. reynaudiana* grown in high and low P patches under heterogeneous and homogenous P distribution for 30 (top panel), 60 (middle panel) and 90 days (bottom panel) where T1, T2 and T3 had high P concentration gradient between patches (0 and 30 mg.kg⁻¹), T5, T6 and T7 had moderate P concentration gradient (7.5 and 30 mg kg⁻¹), T9, T10 and T11 had initial P concentration of 7.5 mg kg⁻¹; i.e. P-enriched patch, and subsequently high P concentration gradient between patches (0 and 30 mg kg⁻¹); and T4, T8 and T12 had homogenous P distribution of 15, 18.5 and 7.5 + 15 mg kg⁻¹, respectively. Values are mean ± SE (n = 3), * indicates significant difference at p < 0.05 and lowercase letters indicate significant difference between treatments.

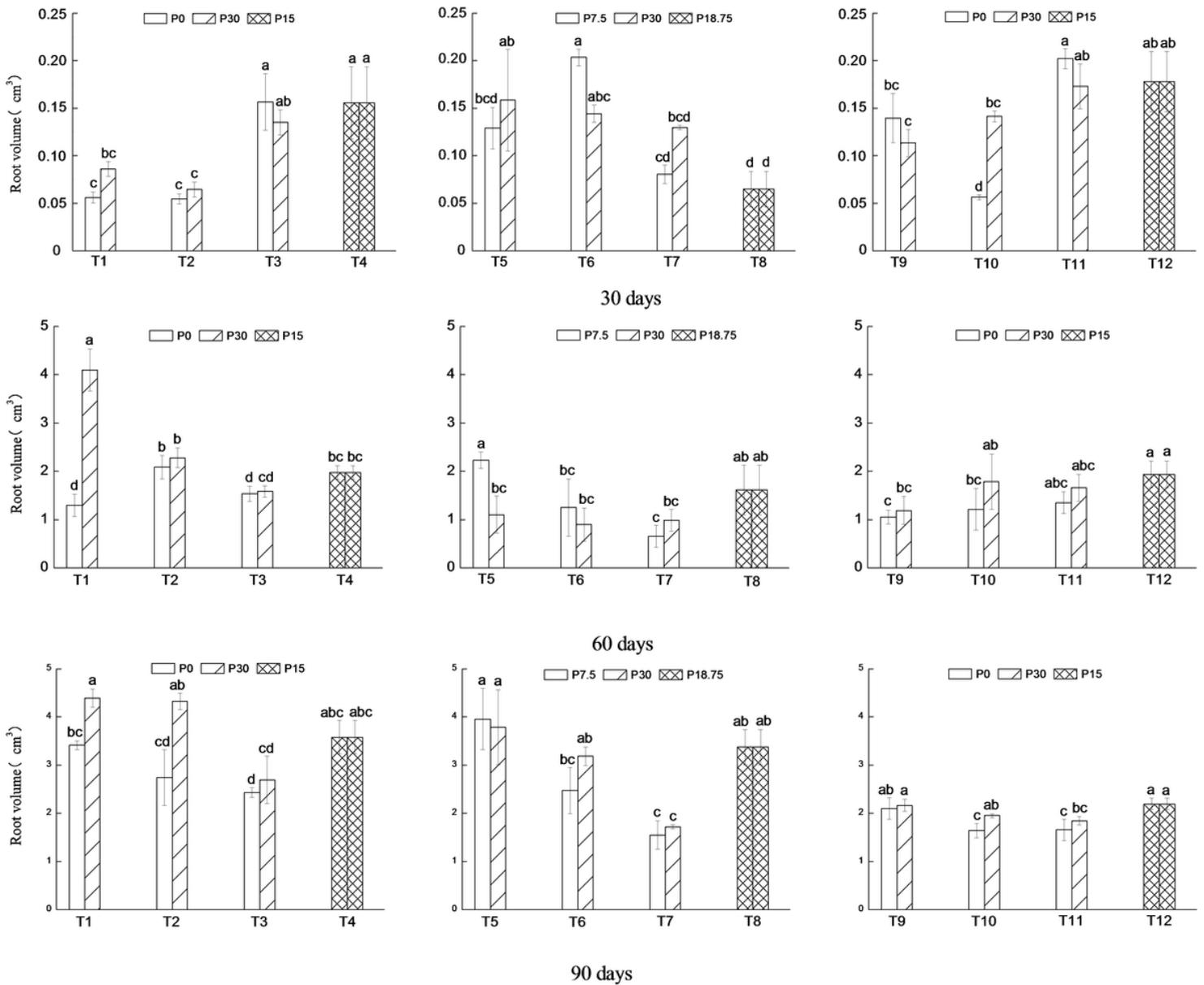


Figure 3

Root volume (cm³) of *N. reynaudiana* grown in high and low P patches under heterogeneous and homogenous P distribution for 30 (top panel), 60 (middle panel) and 90 days (bottom panel) where T1, T2 and T3 had high P concentration gradient between patches (0 and 30 mg.kg⁻¹), T5, T6 and T7 had moderate P concentration gradient (7.5 and 30 mg kg⁻¹), T9, T10 and T11 had initial P concentration of 7.5 mg kg⁻¹; i.e. P-enriched patch, and subsequently high P concentration gradient between patches (0 and 30 mg kg⁻¹); and T4, T8 and T12 had homogenous P distribution of 15, 18.5 and 7.5 + 15 mg kg⁻¹, respectively. Values are mean ± SE (n = 3), * indicates significant difference at p ≤ 0.05 and lowercase letters indicate significant difference between treatments.

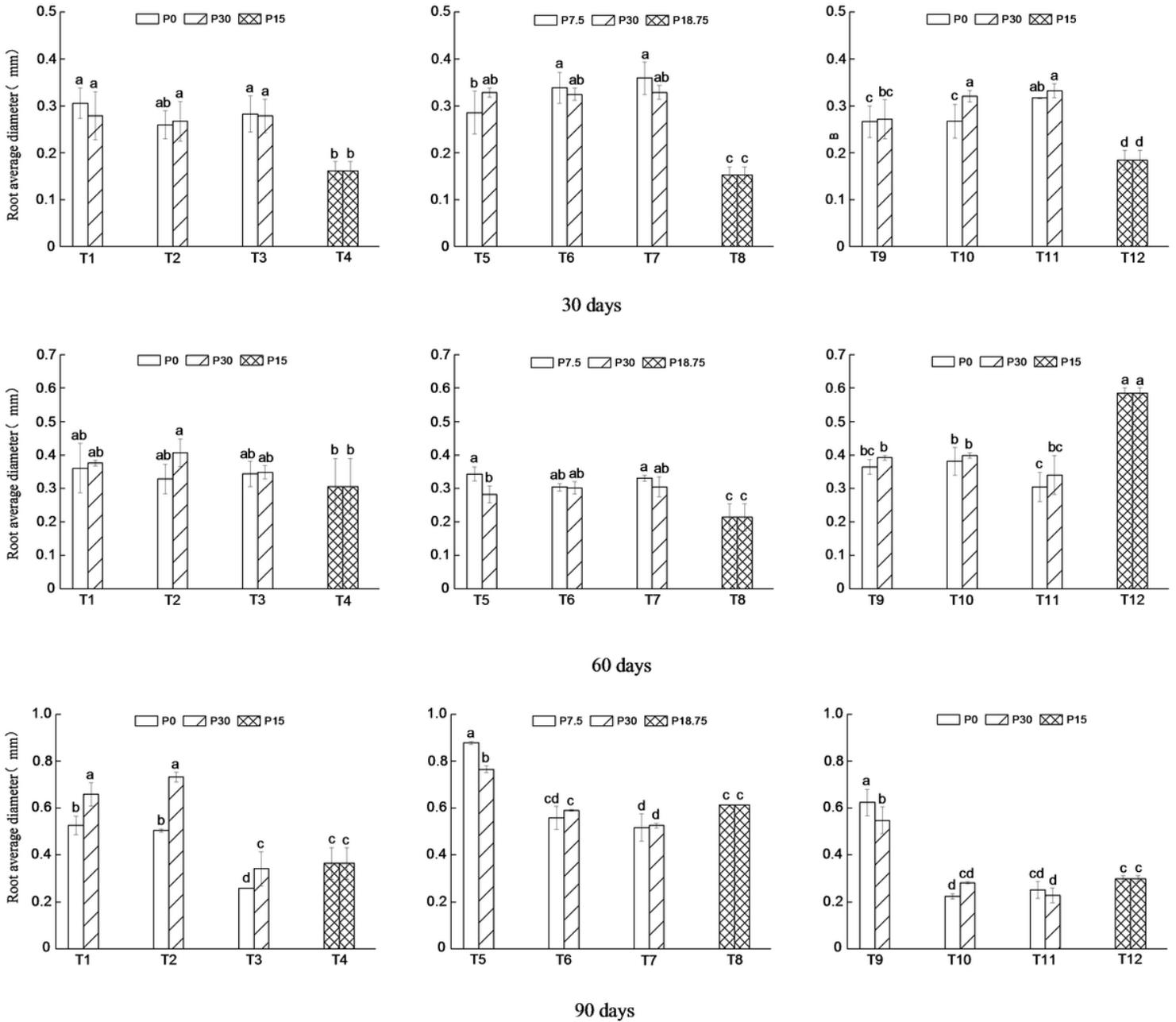


Figure 4

Average root diameter (mm) of *N. reynaudiana* grown in high and low P patches under heterogeneous and homogenous P distribution for 30 (top panel), 60 (middle panel) and 90 days (bottom panel) where T1, T2 and T3 had high P concentration gradient between patches (0 and 30 mg.kg⁻¹), T5, T6 and T7 had moderate P concentration gradient (7.5 and 30 mg kg⁻¹), T9, T10 and T11 had initial P concentration of 7.5 mg kg⁻¹; i.e. P-enriched patch, and subsequently high P concentration gradient between patches (0 and 30 mg kg⁻¹); and T4, T8 and T12 had homogenous P distribution of 15, 18.5 and 7.5 + 15 mg kg⁻¹, respectively. Values are mean ± SE (n = 3), * indicates significant difference at p ≤ 0.05 and lowercase letters indicate significant difference between treatments.

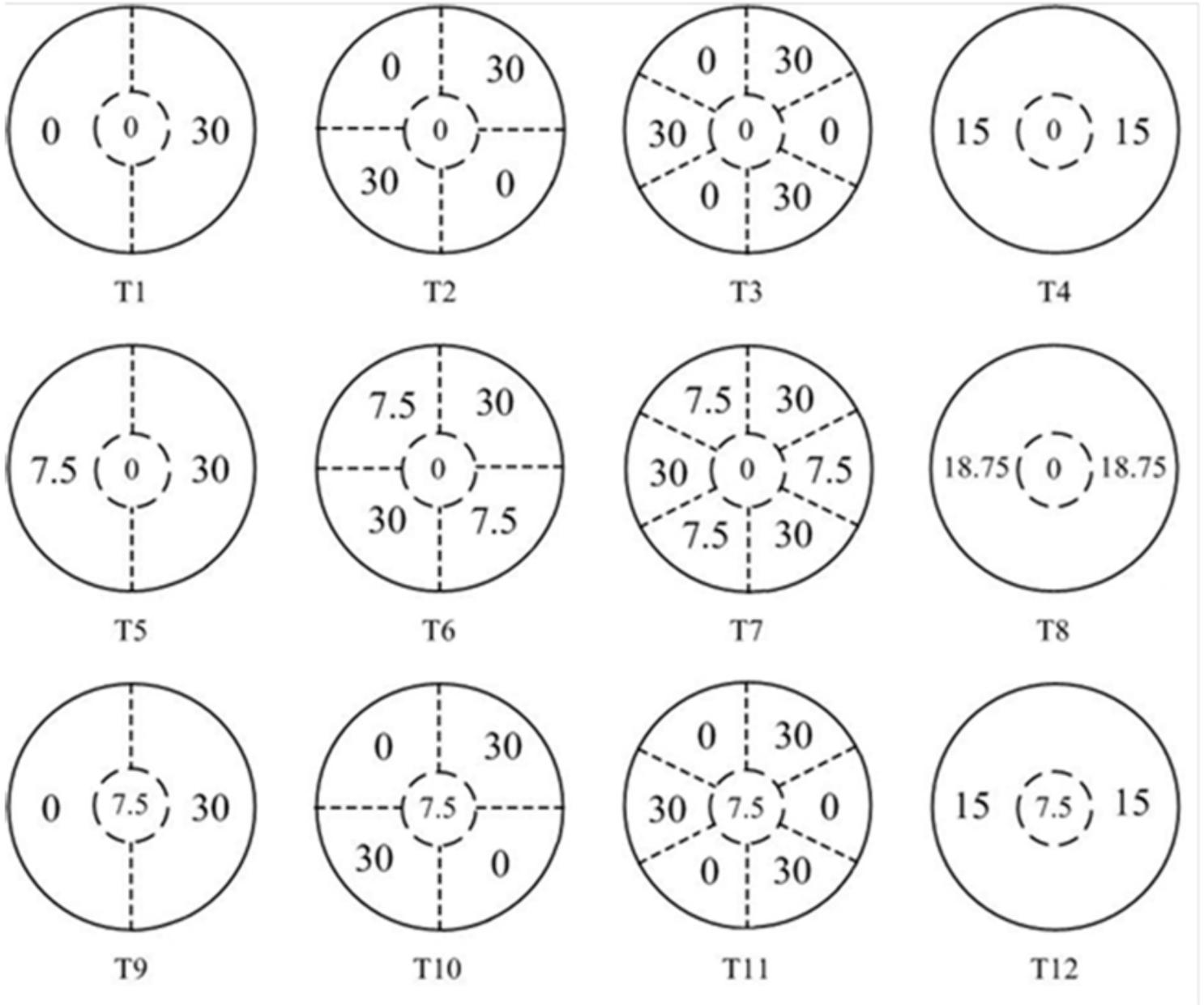


Figure 5

Sketch of the experimental pot used to simulate patchily P distribution, and experimental design showed the size and the amount of P nutrient ($\text{mg} \cdot \text{P} \cdot \text{kg}^{-1}$). The values 0, 7.5, 15, 18.75, 30 showed the amount of P added in each patch. T1, T5, T9 indicated large patch, T2, T6, T10 indicated medium patch, T3, T7, T11 indicated small patch with different P concentrations, and T4, T8, T12 indicated homogeneous P distribution with different P concentration.