Root over-production in heterogeneous nutrient environment has no negative effects on *Zea mays* shoot growth in the field

Hongbo Li[1], Zed Rengel[2], Qinghua Ma[1], Fusuo Zhang[1], Jianbo Shen[1, *]

[1]*College of Resources and Environmental Sciences, Center for Resources, Environment and Food Security, China Agricultural University, Key Laboratory of Plant-Soil Interactions, Ministry of Education, Beijing 100193, P. R. China*

[2]*Soil Science and Plant Nutrition, School of Earth and Environment, The UWA Institute of Agriculture, The University of Western Australia, Crawley, WA 6009, Australia*

*Corresponding author:

Prof. Dr. Jianbo Shen

Department of Plant Nutrition

China Agricultural University

No.2 Yuan-ming-yuan West Road,

Beijing 100193

P. R. China

Phone: +86 10 62732406

Fax: +86 10 62731016

E-mail: jbshen@cau.edu.cn

Abstract

Background and Aims Nutrient patches in soil have a limited life-span, but the long-term costs and benefits of root foraging in agro-ecological systems are poorly understood.

Methods Maize (*Zea mays* L) was grown in homogeneous or heterogeneous nutrient (nitrogen and phosphorus) environments in the field. After patch exhaustion, nutrient supply was switched from heterogeneous to homogeneous (Het1-Hom) or remained heterogeneous in the same location (Het1S) or had a new patch location established (Het1-Het2).

Results Heterogeneous nutrient supply induced fine root proliferation and produced more shoot biomass than homogeneous treatments in the early growth stages. However, local roots still proliferated after nutrient patch exhaustion, resulting in temporary root over-production. In the Het1-Het2 treatment, roots still proliferated in the initial (subsequently depleted) patch, and new roots proliferated in the new patch at 15 days after the nutrient supply changed. Maize did not show decreased shoot biomass or nutrient accumulation because of temporary root over-production, which might have been due to low cost:benefit ratio of fine roots, quick redistribution of nutrients within the plant and/or buffering effect of rhizosphere nutrients.

Conclusions Our data suggest that temporary root over-production has little negative effects on plant growth, especially if plants have large root morphological plasticity.

Key words: costs; nutrient spatial and temporal variability; growth rate; root proliferation; shoot biomass; maize (*Zea mays*)

Introduction

Nutrient distribution in natural soil environments is heterogeneous spatially and temporally. To adapt to such heterogeneous environments, roots often exhibit morphological and/or physiological plasticity to enhance nutrient capture (Farley and Fitter 1999; Hodge 2004; Eissenstat et al. 2015). Lateral root proliferation in the nutrient-rich zone is the most common root morphological response (Robinson 1994, 2001; Liu et al. 2015). However, root proliferation might not be cost-effective if a patch disappears or if a competitor occupies it more rapidly (van Vuuren et al. 1996; Fransen and de Kroon 2001).

Duration of the nutrient pulse had a large impact on plant nutrient capture (Campbell and Grime 1989). In natural environments, however, the peaks of nutrient concentration in localized nutrient-rich patches are often short-lived, lasting no more than 4 weeks (Farley and Fitter 1999; Hodge 2006), especially for nutrients that are relatively mobile in soils (such as nitrogen). This implies that inorganic nutrient patches might dissipate relatively quickly, and plant must respond rapidly with the short-lived fine roots to minimize the construction costs. However, root proliferation in a mobile nutrient patch may be maladaptive if the nutrient moves faster than roots can proliferate, especially when such proliferation retards root growth in other soil zones that may potentially have greater nutrient availability in the future, such as new patches appearing (Lynch 2013). The metabolic costs of maintaining roots in the local zone could be substantial when the nutrient was depleted (Fisher et al. 2002). In a 2-year field experiment, Fransen and de Kroon (2001) found that fast root proliferation and high nutrient uptake within the nutrient

patch could result in increased shoot biomass of *Holcus lanatus* in the short term, but the positive effect was curtailed by rapid patch exhaustion and high carbon cost due to a limited root life-span. These findings suggested that if patches are ephemeral, root over-production may be a major cost to plant growth in the long term. So, understanding the long-term costs of root proliferation in patches with limited duration or in other patches appearing elsewhere, could be important not only to understanding natural ecosystems, but also may help improve nutrient management in the agro-ecological systems.

Heterogeneous nutrient environments are regularly created in agro-ecological systems. Banding fertilizers as the starter fertilizer and side-dressing fertilizer in different crop growth stages is a widely used way to improve fertilizer-use efficiency in modern agricultural production (Bordoli and Mallarino 1998; Vetsch and Randall 2002; Frame et al. 2013; Sangoi et al. 2014). Root responses to banding fertilizers play a crucial role in nutrient capture by crops and thus improve the crop capacity to exploit soil, reducing the reliance on application of fertilizers (Malhi et al. 2001; Shen et al. 2013; Zotarelli et al. 2015). However, banding fertilizers in agricultural soils has an inherent problem: only a part of the root system can capture nutrients from the nutrient-rich zone (Robinson 1994) because roots have to reach the nutrient patches and then proliferate and exploit them (Forde and Lorenzo 2001). This implies that heterogeneous nutrient supply is unlikely to result in all roots being located in “ideal” places regarding their nutrient environment to maximize efficiency of the whole root system throughout the growing season; if this hypothesis is correct, roots still growing in some local places may not be cost-effective to crop growth, especially when the old patch (starter fertilizer) was exhausted and roots

proliferate in the new patches (side-dressing) in intensive agriculture. However, there is little knowledge on dynamics of temporal and spatial variability in root proliferation and nutrient-patch exploration in terms of cost-benefit to crop growth, even though this may be important for sustainable crop production.

Maize (*Zea mays* L.), one of the main crops in China, is plastic in its root morphological responses to soil nutrient heterogeneity. In our previous studies, localized ammonium and phosphorus significantly induced root proliferation and improved shoot dry biomass and nutrient content of maize seedlings (Jing et al. 2010; Li et al. 2012; Ma et al. 2013). However, these positive effects disappeared after exhaustion of available soil nitrogen (N) in the localized patches at 51 days after sowing, while root proliferation lasted longer (Jing et al. 2010, 2012). These results imply that the root proliferation is induced by short-lived plant-available soil N in the nutrient zone, but root temporary over-production may occur when the nutrient-rich zone was depleted and the metabolic costs of the construction and maintenance of these roots may reduce the growth of other roots or the shoot (Lynch 2007). Thus, these findings raise the question of whether root proliferation may lead to a reduction in above-ground growth (expressed as growth rate or biomass) as a result of non-performing "investment" in the nutrient-exhausted patch, and how plants respond to the changing nutrient conditions such as a new nutrient-enriched patch added at another location.

An understanding of the cost of temporary over-production under heterogeneous nutrient environments in the field would clarify the potential negative effects of banding fertilizer in different maize growth stages on crop yields. Based on the current knowledge, the possible effects of altering heterogeneous nutrient supply over the course of plant

113 growth processes are unclear. The objective of the present study was to test whether
114 long-term costs of root over-production have a negative effect on plant growth and
115 nutrient uptake, especially when the nutrient patch location is changed, because benefits of
116 initial root proliferation in a nutrient patch decline and the costs of root proliferation in a
117 new location are relatively high. Our hypothesis is that costs of root over-production in
118 heterogeneous soil environments are limited, meaning that plants have the capacity to
119 quickly adapt to dynamic and complex nutrient environments in soil.

120

121 **Materials and methods**

122

123 Field experiment design

124 A 2-year field experiment was carried out to test the long-term costs of root proliferation
125 in local nutrient-enriched zones on maize growth in an intensive farming system in North
126 China in 2011 and 2012. The experimental site was located at the research station of China
127 Agricultural University in Shangzhuang, North China (40 °N latitude, 116 °E longitude).
128 The soil type in the study site was silt loam with bulk density 1.37 g cm^{-3}. Initial soil test
129 results for the site were pH 7.9 (1:5 soil:water suspension), organic carbon 7.3 g kg^{-1}, total
130 N 0.78 g kg^{-1}, Olsen-P 8.6 mg kg^{-1} and available K 78 mg kg^{-1} in the topsoil layer (0-30
131 cm). Maize (*Zea mays* L. cv. DH661) was sown at density of 100,000 plant ha^{-1}. Plants
132 were arranged in alternating rows with 20-cm and 50-cm width (narrow and wide rows,
133 respectively) (Fig. 1), and the plot size was 4.9 m×10 m. The experiment was set up in a
134 completely randomized block design with four replicates. No irrigation was provided in
135 2011 and 2012 due to sufficient rainfall (Ma et al. 2013, 2014).

There were four fertilizer treatments established in 2011. All treatments had nutrients supplied two times during the whole maize growth cycle: before sowing and 45 days after sowing (DAS) (Table S1). The nutrient treatments were established immediately prior to sowing. For homogeneous nutrient supply, N and P fertilizers (as well as other nutrients) were hand-broadcast and then mixed with soil to the 0-30 cm depth by a rotary cultivator, whereas heterogeneous nutrient supply involved localized application by placing fertilizers 10 cm away from the sowing site and 10 cm below the soil surface. The nutrient treatments (Fig. 1) were: 1) homogeneous supply of N and P before sowing, and N fertilizer supplied homogeneously at 45 DAS (Hom-Hom); 2) N and P supplied heterogeneously (location 1, middle of narrow rows) just before sowing, and then N fertilizer supplied homogeneously at 45 DAS (Het1-Hom); 3) heterogeneous (localized) application of full-year complement of N and P (stable heterogeneous treatment: Het1S) just before sowing at location 1, and 4) N and P supplied heterogeneously the first time (just before sowing) at location 1 and the second time (at 45 DAS) at location 2 (wide rows) (Het1-Het2) (Fig. 1).

In all treatments, the standard fertilizer application rates in this region were as follows (Table S1): the Hom-Hom and Het1-Hom treatments had 130 kg P_2O_5 ha^{-1} (P as single superphosphate) and 60 kg N ha^{-1} (N as urea) applied before sowing, and 120 kg N ha^{-1} (as urea) broadcast at 45 DAS (after soil and plant sampling); the Het1S treatment had 130 kg P_2O_5 ha^{-1} and 180 kg N ha^{-1} (60 N as standard urea + 120 N as controlled-release urea (Osmocote) with 60-day longevity) applied at location 1 before sowing (to avoid excessive root damage that would have been caused by digging in the middle of narrow rows at 45 DAS); the Het1-Het2 treatment had 65 kg P_2O_5 ha^{-1} and 60 kg N ha^{-1} applied at location 1 before sowing, and 120 kg N ha^{-1} and 65 kg P_2O_5 ha^{-1} supplied at two locations 2

159 (half-and-half) at 45 DAS (Fig. 1). All treatments received the same amounts of N and P
160 (Table S1). To avoiding the influence of the soil and root disturbance caused by deep
161 placement of the fertilizer at location 2 in the Het1-Het2 treatment, we also disturbed the
162 soil at location 2 in other three treatments but without fertilizer addition.

163 To further confirm the effect of root proliferation in location 1 on maize growth, based
164 on the treatments applied in 2011, the next year (2012) we eliminated the Het1-Het2
165 treatment and added another the treatment with localized application of 60 kg N ha^{-1} (urea)
166 and 17 kg P_2O_5 ha^{-1} (single superphosphate) with 120 kg N ha^{-1} using organic fertilizer
167 (total N 1.63% w/v and P_2O_5 1.54% w/v), which was denoted Het1SOF. So, there were
168 four treatments in 2012 and all treatments were established at sowing at location 1:
169 Hom-Hom, Het1-Hom, Het1S and Het1SOF.

170

171 Soil and root sampling

172 To assess root proliferation and soil mineral nitrogen concentration, an auger
173 sampling method was used in all treatments at 30, 45, 60 and 75 DAS. Soil cores (10 cm
174 diameter and 15 cm depth) were collected in locations 1 and 2 (Fig. 1). Roots were
175 collected from soil by passing through a 2 mm-diameter mesh and washed clean with
176 water, and then root length was measured by a scanner and the WinRhizo software
177 (Regent Instruments Inc., Canada). The soil from each core was passed through a
178 2-mm-diameter mesh and placed in an icebox for transport to the lab, followed by
179 measuring the soil mineral nitrogen concentration (Nmin): 12 g fresh soil was mixed with
180 100 mL 0.01 M $CaCl_2$ on a shaker (180 rpm) for 1 h at 25°C, and then Nmin was
181 measured by a continuous flow analyser (TRACS 2000 system, Branand Luebbe,

Norderstedt, Germany). About 20 g soil was used to measure soil moisture content by oven drying at 105°C.

Sampling of above-ground plant parts

Six plants per plot were randomly sampled at 30, 45, 60, 75 and 105 DAS. All plant samples were heated at 105°C for 30 min to cease metabolic activity, and were then oven-dried for 72 h at 60°C. Dry samples were weighed, crushed and homogenized; the subsample was digested in a mixture of concentrated H_2SO_4 and H_2O_2. The digests were analyzed for total N using the Kjeldahl method and for total P using the molybdovanado phosphate method (Johnson and Ulrich, 1959). In each treatment, 20 plants were randomly selected to measure chlorophyll content in the youngest fully-developed leaves using a chlorophyll meter (SPAD-502, Minolta, Osaka, Japan).

Statistics

In order to determine the rate of root length increase within the nutrient patch zone, and how it was affected by changing nutrient patches, root absolute growth rate (AGR) and relative growth rate (RGR) were calculated for locations 1 and 2 in all treatments and for all four harvests in 2011 separately, using the following formulas:

$$AGR = \frac{RLD_{t+1} - RLD_t}{T_{t+1} - T_t}$$

$$RGR = \frac{\ln RLD_{t+1} - \ln RLD_t}{T_{t+1} - T_t}$$

where RLD denotes the values of the root length density in location 1 or 2 (see Fig. 1); T denotes time in days between the two harvest periods (t followed by t+1).

Repeated-measure analysis of variance (ANOVA) was conducted to test the effects of nutrient treatments on soil, root (at four harvests) and shoot (at five harvests) variables measured in each 2011 and 2012. ANOVA was conducted using the SAS statistical software (SAS Inst., 2001). Significant difference among means was determined by LSD at the $P \leq 0.05$ probability level.

Results

Plant-available soil N

In 2011, plant-available soil N (soil Nmin) concentration at location 1 was significantly influenced by time and nutrient treatments (both $P < 0.001$) before the second nutrient application (sampling at 30 and 45 DAS); however, after the second nutrient application (sampling at 60 and 75 DAS), only the time factor significantly influenced soil Nmin concentration (Table S2). At location 2, soil Nmin concentration was only significantly influenced by the nutrient treatment ($P < 0.001$) before the second nutrient application; afterwards, both main factors and their interaction significantly influenced soil Nmin concentration (all $P < 0.001$).

At 30 DAS, soil Nmin concentration was higher in the local nutrient patch (location 1), and significantly lower in location 2 (no nutrient application yet), than that in the homogeneous treatment (Fig. 2a). With duration of plant growth, soil nutrient exhaustion

occurred quickly in the patch at location 1 until no significant difference existed among the treatments at 60 and 75 DAS, with a declining trend being slower in the Het1S treatment (eg. at 45 DAS) than the other heterogeneous nutrient treatments (Fig. 2b, 2c, 2d). Unsurprisingly, the soil Nmin concentration was higher in the homogeneous than heterogeneous nutrient treatments in location 2 before the second nutrient application (45 DAS). After the second nutrient application, the soil Nmin concentration was significantly higher in the Het1-Het2 than other treatments in location 2 at 60 DAS (Fig. 2c), and the trend was also maintained at 75 DAS, although the soil Nmin concentration in all treatments was relatively low.

In 2012, soil Nmin concentration at location 1 was significantly influenced by the nutrient treatment ($P < 0.001$) and the interaction of time and the nutrient treatment ($P < 0.05$) before the second nutrient application; however, after the second nutrient application (sampling at 60 and 75 DAS), only the time factor significantly influenced soil Nmin concentration (Table S5). These results implied that soil Nmin was influenced by the nutrient supply patterns only in the early growth stage of maize. The change trend of average values was similar with that in 2011. At 30 DAS, soil Nmin concentration was much higher in the local nutrient patch (especially in the Het1S treatment) than that in the homogeneous treatment. With duration of plant growth (at 45 DAS), soil nutrient exhaustion occurred in the patch at location 1; there was no significant difference between the Het1-Hom and Hom-Hom treatments, whereas the Het1S and Het1SOF treatments still remained with higher soil Nmin until 60 and 75 DAS (Table 1).

Root growth

247 Both before and after the second nutrient application in 2011, time and the nutrient
248 treatment had highly significant effects on root length density in the patch zone (location 1)
249 (before the second nutrient application: $P < 0.01$ and $P < 0.001$ for the time effect and the
250 nutrient treatment, respectively; after the second nutrient application: $P < 0.001$ and $P <$
251 0.01, respectively). There was no significant interactive effect of time and the nutrient
252 treatment. At location 2, only the time factor before the second nutrient application ($P <$
253 0.05) and the nutrient treatment after the nutrient second nutrient application ($P < 0.001$)
254 had significant effects on root length density (Table S2). Heterogeneous nutrient supply
255 significantly stimulated root proliferation in the soil zone where the nutrients were locally
256 applied (location 1, Fig. 3). The root length density in location 1 was significantly greater
257 in the heterogeneous than homogeneous nutrient treatment at 30 DAS (403-466%), 45
258 DAS (780-887%), 60 DAS (585-815%) and 75 DAS (152-662%). There was slightly
259 higher root length density in location 2 in the homogeneous than heterogeneous nutrient
260 treatment at 30 DAS (Fig. 3a). In the Het1-Het2 treatment at 60 DAS (15 days after
261 second nutrient application), there was a significant effect of heterogeneous nutrient
262 supply on root proliferation in location 2 (Fig. 3c), and the significant effect lasted until 75
263 DAS (Fig. 3d). The root length density in locations 1 and 2 was not different among the
264 treatments at 105 DAS (data not shown). Heterogeneous nutrient supply significantly
265 stimulated proliferation of fine roots in the nutrient-rich zone. In location 1, the proportion
266 of root length with diameter ≤0.2 mm was 19% greater, and of diameter ≤0.4 mm 26%
267 greater, in the Het1-Hom than the Hom-Hom treatments at 30 DAS (Fig. S1).

268 Time and the nutrient treatment influenced root length density throughout growth in
269 2012 (Table S5). In all growth stages, the root length density was always higher in the

patch zone of the heterogeneous nutrient treatments than in the Hom-Hom treatment. Although the root length density was reduced at 75 compared with 60 DAS, it was much higher in the heterogeneous than homogeneous treatments (Table 1).

Root growth rate

In 2011, the root absolute growth rate (AGR) in location 1 was significantly influenced only by the nutrient treatment ($P < 0.001$) before the second nutrient application, and was influenced only by time ($P < 0.0001$) after the second nutrient application. In location 2, neither the nutrient treatment nor time significantly influenced AGR before the second nutrient application. In contrast, after the second nutrient application, both the nutrient treatment and time had significant effects on AGR ($P < 0.01$ and $P < 0.05$, respectively). No factor had significant influence on root relative growth rate (RGR) in location 1 after the second nutrient application. In contrast, RGR in location 2 after the second nutrient application was significantly influenced by the nutrient treatment, time and their interaction ($P \leqslant 0.05$, $P < 0.001$ and $P < 0.01$, respectively) (Table S3).

Heterogeneous nutrient supply significantly increased root AGR in the nutrient patch zone (location 1), especially before the second nutrient application (Fig. 4a, 4b). At 60 DAS (ie. after the second nutrient application), only the Het1S treatment had AGR in location 1 higher than the homogeneous nutrient treatment (Fig. 4c). At 75 DAS, root proliferation in location 1 stopped, and significant negative AGR occurred (Fig. 4d). Heterogeneous nutrient supply at location 2 also significantly increased root AGR

compared with the Hom-Hom treatment at 60 DAS, with no significant influence at 75 DAS.

Root RGR in location 1 was not significantly influenced by heterogeneous nutrient supply at 45 and 60 DAS (Fig. 4e). The RGR trend was similar to that of AGR at 75 DAS, with only the Het1-Hom treatment having a significantly lower negative RGR than homogeneous nutrient supply. The second nutrient application improved root RGR in the Het1-Het2 treatment in location 2 at 60 DAS, and the positive effect disappeared at 75 DAS (Fig. 4f, 4g).

Plant biomass and nutrient content

In 2011, nutrient treatment had a significant effect on shoot biomass before the second nutrient application ($P < 0.01$), but the effect was non-significant after the second nutrient application (Table S4). Unsurprisingly, there was a significant time effect on shoot biomass ($P < 0.001$), and there was no significant interactions between time and the nutrient treatment. Similar patterns were observed in plant N and P contents (Table S4).

Shoot dry weight was higher in the heterogeneous nutrient environments before the second nutrient application. Shoot dry weight increased by 63-95% at 30 DAS and 36-47% at 45 DAS in the heterogeneous compared with the homogeneous nutrient treatment (Fig. 5a). After the second nutrient application, shoot dry weight was significantly higher in the Het1S than Hom-Hom treatment at 60 and 75 DAS, whereas there was no significant difference between the other heterogeneous nutrient treatments (Het1-Hom and Het1-Het2) and the Hom-Hom treatment. No significant difference was observed among the

313 treatments at 105 DAS (Fig. 5d). A same trend was also observed in plant N and P
314 contents (Fig. 5).

315 In 2012, the nutrient treatment and time had a significant effect on shoot biomass and
316 N content throughout growth stage (Table S6). Shoot dry weight and nutrient content were
317 higher in the heterogeneous nutrient environments than the homogeneous treatment before
318 the second nutrient application. After the second nutrient application, there was no
319 significant difference in shoot dry weight and nutrient content between the Het1-Hom and
320 Hom-Hom treatments at 60 DAS, whereas the Het1S and especially Het1SOF treatments
321 were higher than the Hom-Hom treatment until the final harvest at 105 DAS (Table 1).
322 The heterogeneous nutrient supply significantly increased shoot N concentration and leaf
323 chlorophyll content (SPAD value) at 30 DAS and 45 DAS; however, the difference
324 disappeared after the second nutrient application (60 and 75 DAS). In contrast, the shoot P
325 concentration was little influenced by the nutrient supply patterns (Table S7).

326

327 **Discussion**

328

329 Our results indicated that dynamic changes in root length lagged behind changes in
330 nutrient supply under heterogeneous environments. Firstly, heterogeneous nutrient supply
331 induced maize root proliferation in the local nutrient-rich zone, and root length density
332 was maintained at a high level over time after the patch was exhausted at 60 DAS and later.
333 Secondly, the root proliferation in the initial location did not stop upon patch exhaustion

and a new patch application elicited root proliferation. Despite the temporary root over-production, maize did not exhibit lower shoot biomass or nutrient uptake.

Root growth

Root proliferation within nutrient patches is one of the most obvious responses in the heterogeneous nutrient environments (Kembel and Cahill 2005; Pinno and Wilson 2013). In the present study, high soil mineral N concentration induced root proliferation in the nutrient patch zone in the heterogeneous nutrient treatments; given that most of these roots had a diameter of ≤0.2 mm (Fig. S1), they had a high specific root length. Roots with high specific root length have a more rapid rate of root proliferation and higher nutrient-uptake efficiency in the patch than coarse roots (Eissenstat 1991; Li et al. 2014; Liu et al. 2015). These advantages of fine root proliferation in the nutrient-rich zone contributed to increased maize shoot growth and nutrient content compared with the Hom-Hom treatment.

This positive effect of heterogeneous N and P supply on root proliferation was mainly mediated by soil Nmin concentration (Jing et al. 2012). In the present study, root proliferation either increased or was maintained at a high level in the initial localized nutrient zone, even when the patch was depleted of Nmin in the heterogeneous nutrient treatments at 60 DAS (Figs. 2, 3 and 4; Table 1). In contrast, soil Nmin exhaustion was reduced and root proliferation was prolonged in the stable heterogeneous treatment (Het1S and Het1SOF), indicating an extended root response to the patch relative to nutrient exhaustion at the local fertilization site. Similar results on root responses to organic patches were observed in other studies (Hodge et al. 1999, 2000; Hodge 2001), which

showed that root proliferation in the patch zone would enhance microbial populations, and an increase in microbial biomass and turnover of microbial biomass may potentially result in a secondary nutrient-rich patch long after the original patch was exhausted (Hodge 2006). However, in the present study, we did not detect an increase in soil Nmin after the organic patch was exhausted in 2012 (Table 1). Thus, the root proliferation may be due to (i) concentration of other nutrients (such as P) still being high in the patch zone or (ii) a temporal hysteresis in the root response to variable nutrient environments.

Collectively, these results indicated that root proliferation could be induced by heterogeneous nutrient supply, while localized peaks in nutrient concentration in the field could be shorter-lived than the life span of roots (Jansen et al. 2006). Hence, temporary root over-production occurred in the field with banded fertilizer supply in the present as well as in previous studies (Jing et al. 2010, 2012; Ma et al. 2013).

Costs of temporary root over-production

In the present study, root over-production occurred in the heterogeneous nutrient treatments because large root length was still maintained after the nutrient patch was depleted and plants had to invest in root growth in a new nutrient-rich location. However, no long-term differences were found in shoot biomass or shoot nutrient content after the second nutrient application. We suggest three explanations for the lack of a negative impact of carbon and energy costs of building a proliferated root system.

Firstly, roots proliferating in the nutrient-rich zone were fine (diameter $\leq$0.2 mm), with a relatively high nutrient-uptake rate due to a large surface area of contact with soil per

379 unit of carbon invested; hence, the costs of temporary root over-production were relatively
380 small and did not affect shoot growth that was supported by root proliferation. Fine roots
381 can be employed effectively to exploit the resources within short-lived patches (Eissenstat
382 et al. 2000; Hodge 2006). Therefore, root proliferation in the nutrient-rich zone increased
383 maize shoot growth and nutrient content in the short term (Fig. 5a, 5b, 5c). With continued
384 plant growth, nutrients in the patch were exhausted; lowering root nutrient uptake
385 efficiency as well as root respiration (Bouma et al. 2001; Volder et al. 2005) would
386 minimize the cost of temporary root over-production (from 45 DAS to 75 DAS, Fig. 3).
387 On the other hand, once roots show decreasing efficiency, plants may stop investing in
388 defensive compounds for these roots, leaving them vulnerable to pathogens (Eissenstat
389 and Volder 2005), which could accelerate fine root deaths (Fig. 4d), reducing maintenance
390 costs.

391 Secondly, the cost of root over-production was alleviated by plant capacity to capture
392 residual soil N and other nutrients (such as P and Zn) in the rhizosphere. Although the soil
393 N concentration at location 1 was much lower at 75 DAS than in previous growth stages,
394 the N concentration of the patch was still about 2 times higher than the background N status
395 of the soil to which the remainder of the root system was exposed in the Het1S treatment
396 (Fig. 2d). In addition, root proliferation in the patch may be ammonium-dependent, which
397 could induce rhizosphere acidification and improve the availability of P (Jing et al. 2010,
398 2012) and Zn (Ma et al. 2014) in the local zone. Therefore, the newly proliferated roots in
399 the patch zone can still capture some nutrients for plant growth to avoid grossly
400 under-performing “investment”.

401 Thirdly, the cost of temporary root over-production could be counteracted by benefits
402 already accumulated during root proliferation. In the present study, shoot biomass as well
403 as shoot N concentration and leaf chlorophyll content were significantly higher in the
404 heterogeneous than the stable homogeneous treatment at the early growth stages (Table
405 S7). However, shoot growth rate and shoot N concentration decreased after the nutrient
406 supply pattern changed, and no difference was found among the treatments (Fig.5, Table
407 S7). We may thus assume that N that was initially taken up or stored in plants in the
408 heterogeneous environments was redistributed to ensure continued shoot growth when
409 costs of root over-production started to increase. Indeed, luxury consumption is largely
410 considered a bet-hedging strategy for temporal variation in resource supply (van Wijk et al.
411 2003). Our results may suggest the temporary storage of nutrients at the early stages, when
412 luxury consumption was possible, and redistribution of these nutrients later on to buffer
413 the costs of root over-production (cf. Jansen et al. 2006).

414

415 **Conclusions**

416 Heterogeneous nutrient supply could induce fine root proliferation and improve maize
417 shoot biomass growth in the short term. However, the advantage of large shoot biomass
418 and nutrient content was reduced over time by root over-production, resulting in no
419 difference in shoot biomass between homogeneous and heterogeneous nutrient supply in
420 the long term. The costs of temporary root over-production in the localized nutrient-rich
421 zones did not have a negative effect on maize growth because of relatively low
422 cost:benefit ratio of fine roots exploring the nutrient-rich zone. The plant capacity to
423 continue taking nutrients in the depleted patch (albeit at reduced rates) and redistribution

424 of stored nutrient reserves could allow for flexibility in adaptation to varied soil
425 environments. Although there is inherent potential weakness in banding fertilizers in
426 modern agriculture, our results suggest no negative effects of temporary root
427 over-production on crop growth, especially for the crop species with high root
428 morphological plasticity.

429

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431

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Figure legends:

Fig. 1 Nutrient placement in various treatments in the field experiment. Hom, homogeneous (broadcast) nutrient supply; Het, heterogeneous (localized) nutrient supply. Maize was arranged in alternating rows with 20-cm and 50-cm width (narrow and wide rows, respectively). The localized nutrients were applied at locations 1 (narrow rows) and 2 (wide rows) with a distance of 10 cm away from the sowing site and 10 cm below the soil surface. To avoid potential root damage in location 1 that could have occurred had we dug in the middle of narrow rows at 45 DAS, all nutrients in the Het1S treatment were heterogeneously supplied in a single application at location 1 using standard urea (N) and superphosphate (P) together with controlled-release (60-day longevity) urea fertilizer before sowing. The arrow denotes a switch in nutrient supply (top part = nutrient application before sowing, bottom part = nutrient application at 45 days after sowing). The white cycles denote standard urea, the grey cycles denote superphosphate and the black cycles denote controlled-release urea.

Fig. 2 Soil Nmin at locations 1 and 2 at 30 (a) and 45 (b) days after sowing (DAS) as well as 15 and 30 days after the second nutrient application (60 and 75 DAS, respectively; c and d). The treatment codes indicate nutrient supply established at sowing-after the second application. Hom, homogeneous nutrient supply; Het, heterogeneous nutrient supply (see also Fig. 1 and Table S1). The localized nutrients were applied at locations 1 and 2. Values are means of four replicates +standard error (SE). Different letters in each graph denote significant difference ($P \leq 0.05$) among the treatments at the same location and the same sampling time.

562

563 **Fig. 3** Root length density at locations 1 and 2 at 30 (a) and 45 (b) days after sowing (DAS)
564 as well as 15 and 30 days after the second nutrient application (60 and 75 DAS,
565 respectively; c and d). The treatment codes indicate nutrient supply established at
566 sowing-after the second application. Hom, homogeneous nutrient supply; Het,
567 heterogeneous nutrient supply (see also Fig. 1 and Table S1). The localized nutrients were
568 applied at locations 1 and 2. Values are means of four replicates +standard error (SE).
569 Different letters in each graph denote significant difference ($P \leq 0.05$) among the
570 treatments at the same location and the same harvest time.

571

572 **Fig. 4** Root absolute growth rate (AGR; a-d) and relative growth rate (RGR; e-g) at
573 locations 1 and 2 at 30 (a) and 45 (b, e) days after sowing (DAS) as well as 15 (c, f) and
574 30 (d, g) days after the second nutrient application (60 and 75 DAS, respectively). The
575 treatment codes indicate nutrient supply established at sowing-after the second nutrient
576 application. Hom, homogeneous nutrient supply; Het, heterogeneous nutrient supply (see
577 also Fig. 1 and Table S1). The localized nutrients were applied at locations 1 and 2. Values
578 are means of four replicates +standard error (SE). Different letters in each graph denote
579 significant difference ($P \leq 0.05$) among the treatments at the same location and the same
580 harvest time.

581

582 **Fig. 5** Shoot biomass and nutrient content before (a, b and c) [30 (grey bars) and 45 days
583 after sowing (DAS) (grey + white bars)] and after the second nutrient application (d, e and

584 f) [60 (black bars), 75 (black + grey bars) and 105 DAS (black + grey + white bars)]. The
585 treatment codes indicate nutrient supply established at sowing-after the second application.
586 Hom, homogeneous nutrient supply; Het, heterogeneous nutrient supply (see also Fig. 1
587 and Table S1). The localized nutrients were applied at locations 1 and 2. Values are means
588 of four replicates +standard error (SE). Different letters in each graph denote significant
589 difference ($P \leq 0.05$) among the treatments at the same harvest period.

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592 **Fig. 1**

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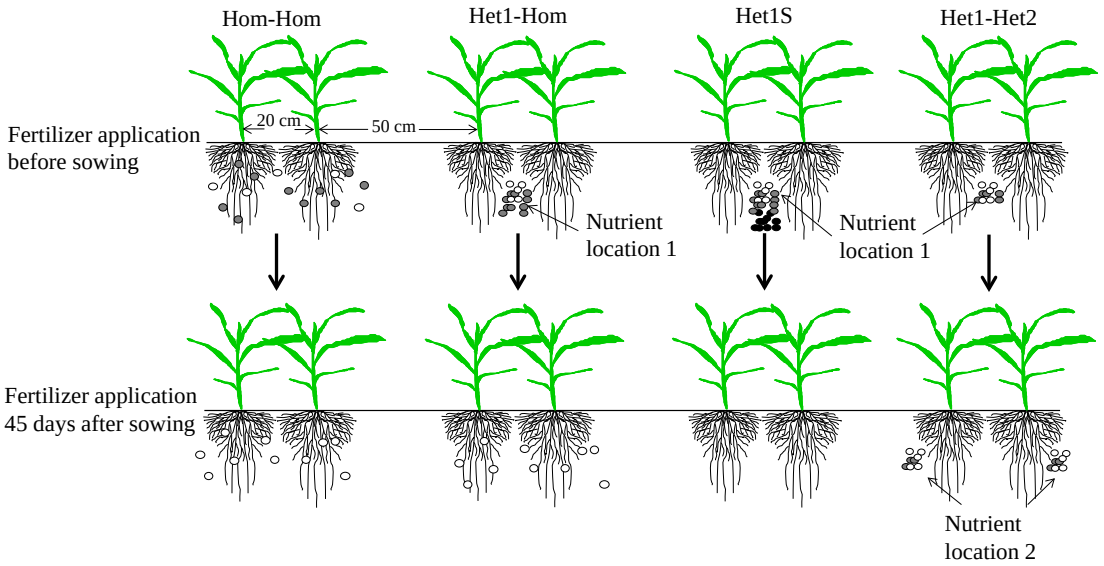

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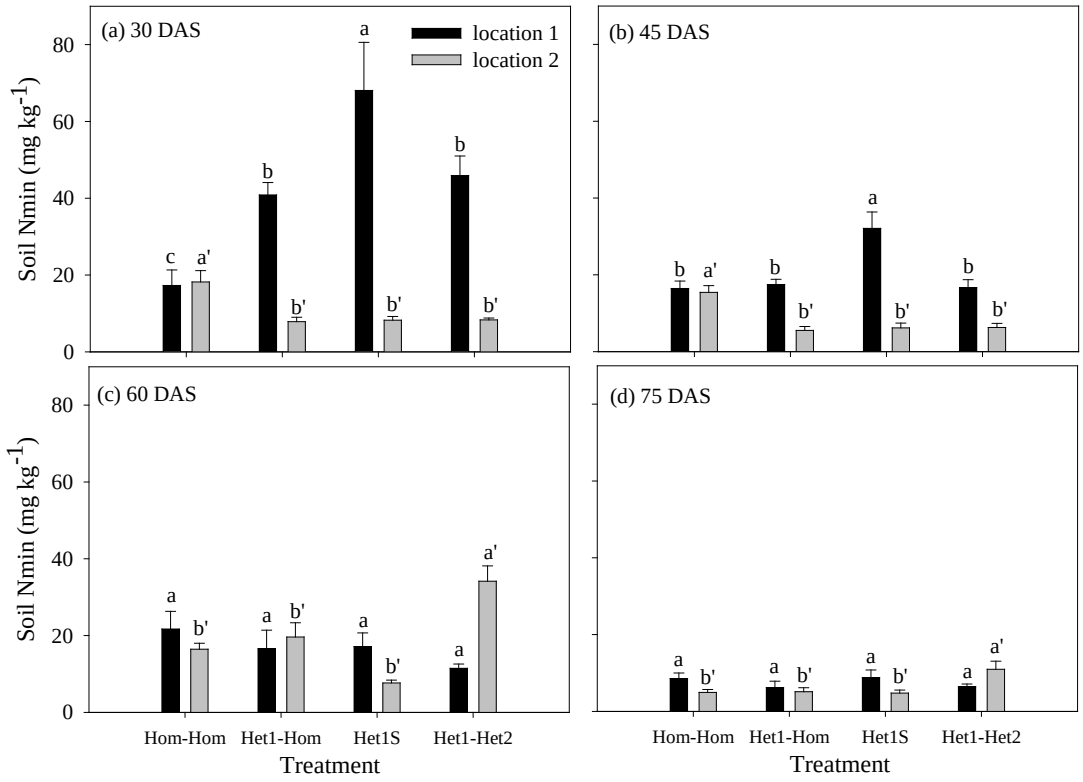
596 **Fig. 2**



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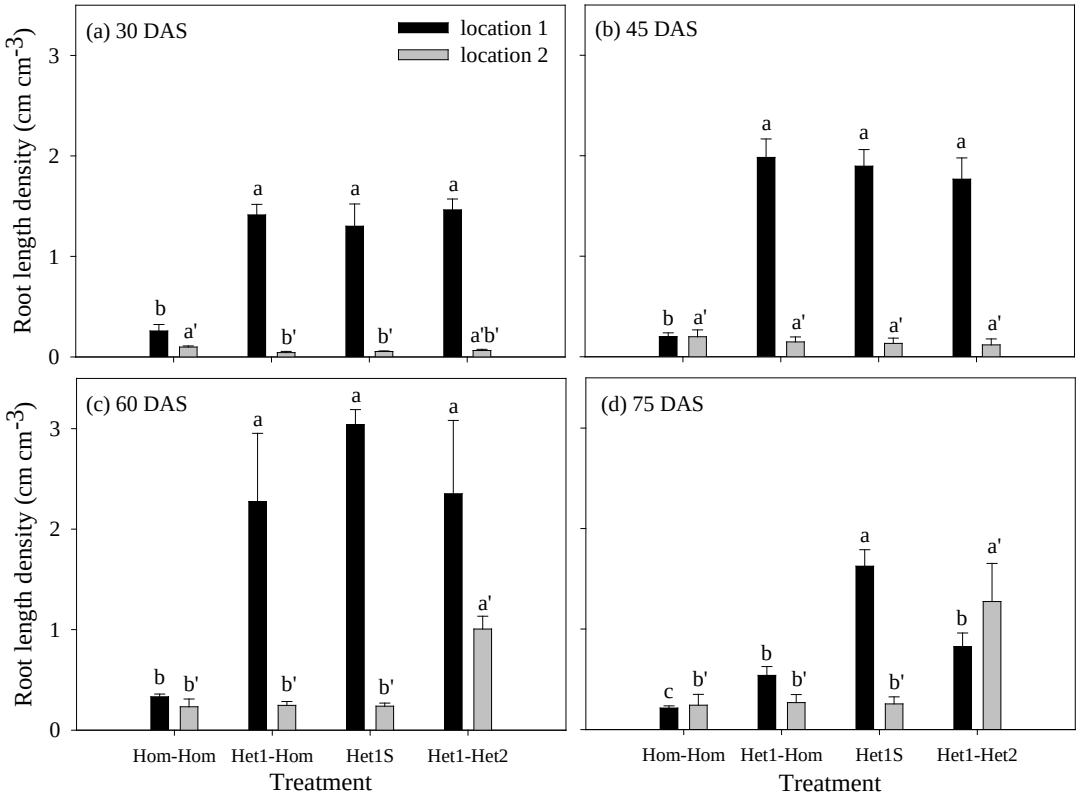
599 **Fig. 3**



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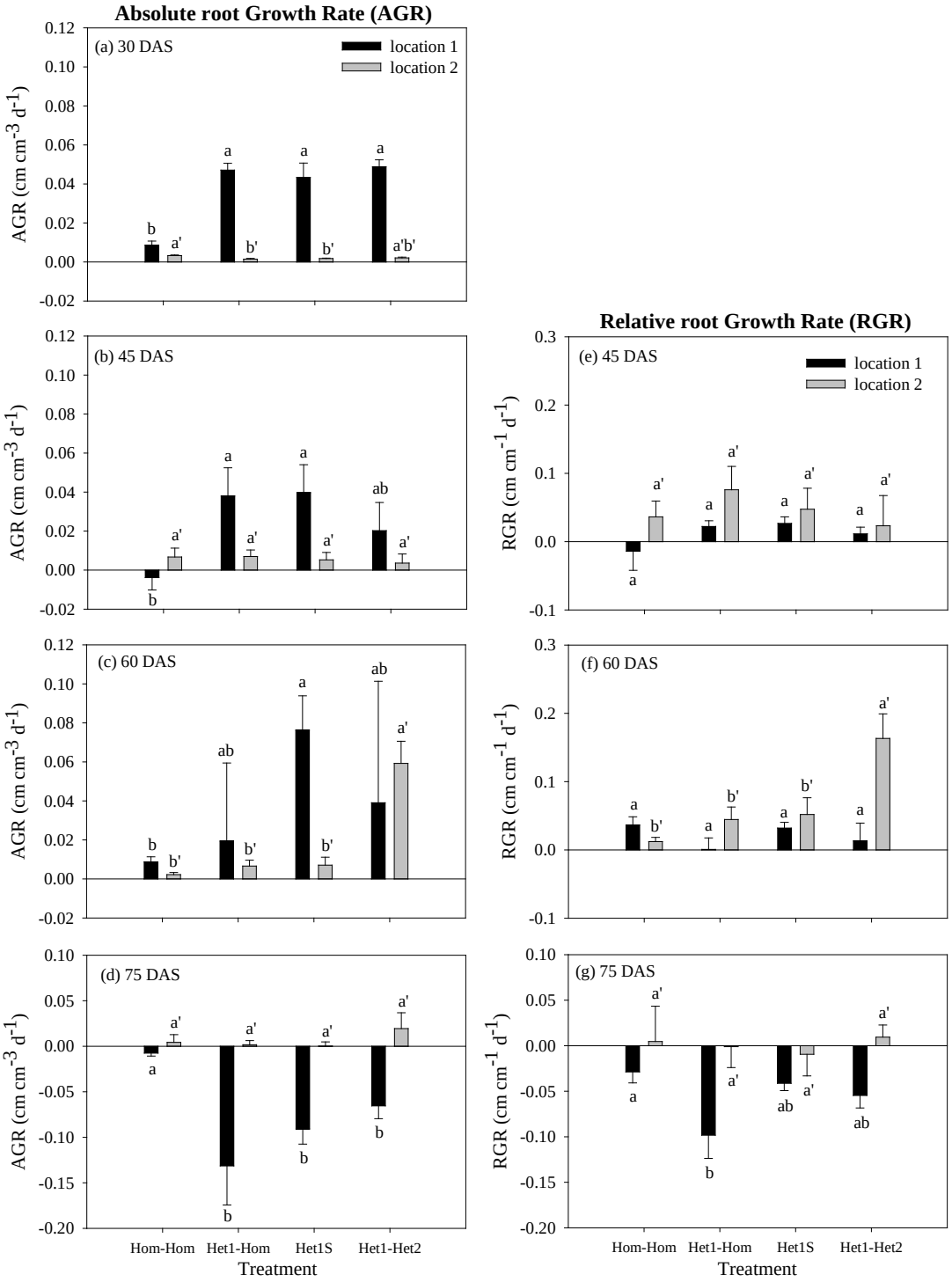

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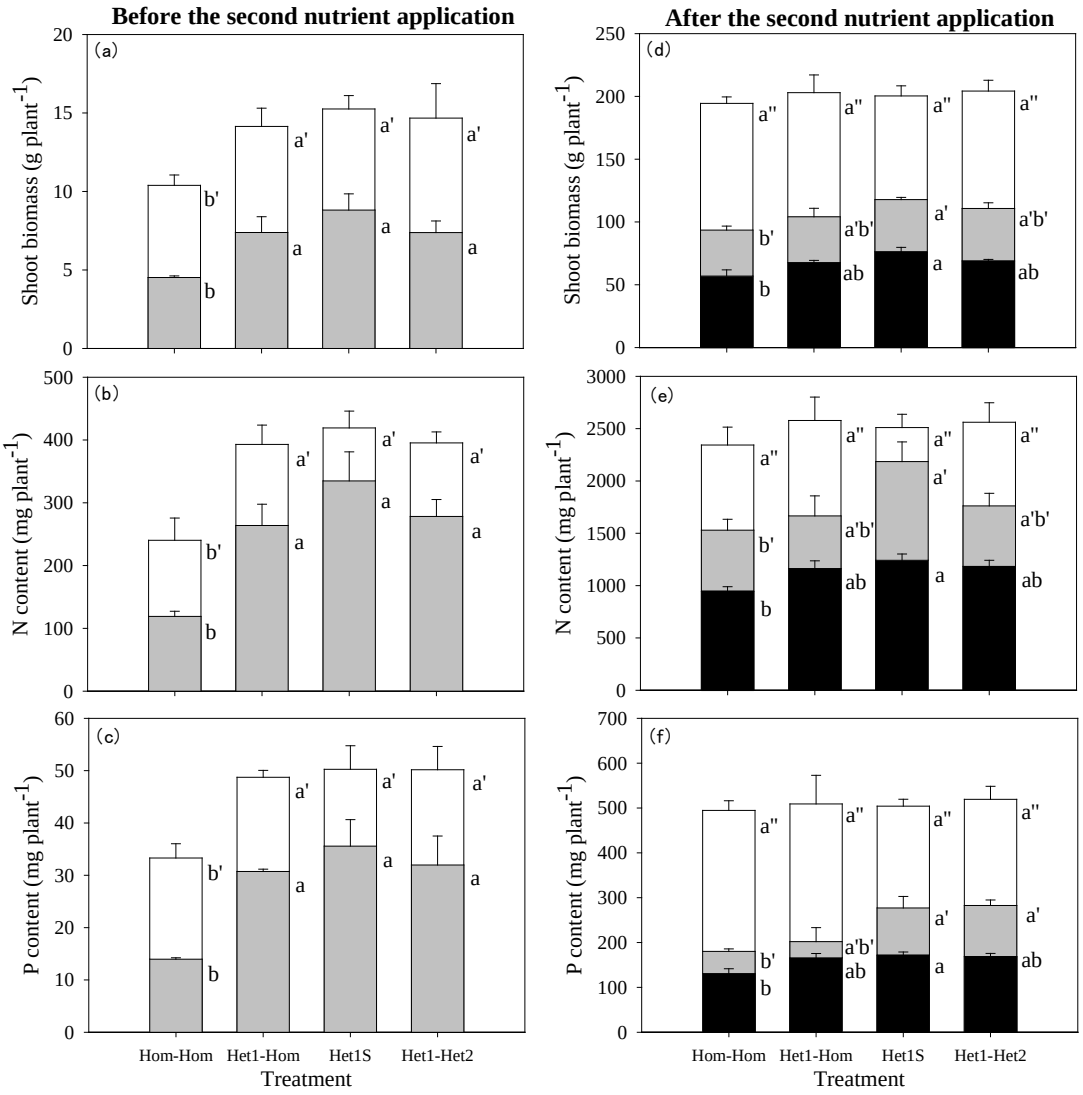
605 **Fig. 5**



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609 **Table 1** Soil Nmin (mg kg^{-1}) and root length density (RLD, cm cm^{-3}) within the patch
610 zone, shoot biomass (g $plant^{-1}$) and nutrient content (mg $plant^{-1}$) of maize in different
611 growth stages in 2012. Hom, homogeneous nutrient supply; Het, heterogeneous nutrient
612 supply (see also Fig. 1 and Table S1). The localized nutrients were applied at location 1
613 (Fig. 1). Values are means of four replicates ±standard error (SE). Different letters in each
614 column for each variable denote significant difference ($P \leq 0.05$) among the treatments at
615 the same harvest time.
616

		Growth stage (DAS)				
Variables		30	45	60	75	105
Soil Nmin (mg kg^{-1})	Hom-Hom	19±3c	16±5c	23±5a	7±1a	-
	Het1-Hom	46±4b	20±6bc	18±3a	7±2a	-
	Het1S	87±16a	110±3a	20±4a	10±2a	-
	Het1SOF	51±5b	37±3b	13±1a	8±3a	-
RLD (cm cm^{-3})	Hom-Hom	0.18±0.03b	0.36±0.07b	0.64±0.09b	0.4±0.1b	-
	Het1-Hom	1.4±0.2a	1.4±0.2a	2.1±0.1a	1.2±0.1a	-
	Het1S	1.1±0.0a	1.4±0.1a	2.7±0.2a	1.9±0.2a	-
	Het1SOF	1.0±0.1a	1.4±0.1a	2.5±0.2a	1.5±0.1a	-
Shoot biomass (g $plant^{-1}$)	Hom-Hom	1.4±0.0b	15±0.2d	72±0.5b	112±1b	228±4a
	Het1-Hom	2.5±0.2a	16±0.1bc	75±1ab	119±2ab	231±3a
	Het1S	2.5±0.2a	17±0.6ab	76±1a	119±3a	228±3a
	Het1SOF	2.4±0.2a	19±0.7a	81±2a	120±2a	237±3a
Shoot N content (mg $plant^{-1}$)	Hom-Hom	43±2b	414±17b	1128±61b	1763±36b	2796±88a
	Het1-Hom	86±7a	489±17a	1251±141ab	1988±55ab	2876±60a
	Het1S	90±7a	512±59a	1283±23ab	1906±57ab	2788±93a
	Het1SOF	81±5a	526±11a	1421±48a	1986±59a	2973±54a
Shoot P content (mg $plant^{-1}$)	Hom-Hom	5.6±0.4b	47±1b	220±3b	280±1b	472±18a
	Het1-Hom	9.4±1a	55±0a	245±11ab	294±10ab	500±58a
	Het1S	9.9±1.5a	57±6a	249±2a	296±9ab	482±28a
	Het1SOF	9.1±0.4a	58±1a	258±12a	301±7a	492±21a

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