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DO HETEROGENEOUS NUTRIENT PATCH SCALE AND DISTRIBUTION ORDER IN THE HABITATS ALWAYS INFLUENCE ON THE BIOPHYSICAL CHARACTERISTICS OF ZOYSIA JAPONICA?

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Abstract

Previous studies have shown that small scale resource heterogeneity may strongly influence plant fitness and many ecological processes. Clonal plant species performed better under various heterogeneous environments compared with homogeneous ones. However, the patch scale and the distribution order in the habitats may affect the clonal growth and has remained unclearly elucidated. We used typical stoloniferous clonal plant *Zoysia japonica* as study material, through analyzing the performance of its clones in the heterogeneous environments with different patch sizes (where amount of nutrient supply was same to all heterogeneous treatments with different patch levels). Levels of patch soil nutrients and patch distribution orders in the habitats, comparing with the homogeneous ones, aiming to test the hypothesis that patch scale and distribution order in the habitats always affect the performance of clonal growth of *Z. japonica*. We did not find significant differences ($p \geq 0.05$) in the total biomass of clonal growth of the species under different heterogeneous conditions, whereas we found significant difference between heterogeneous and homogeneous treatments,total biomass of clonal growth under heterogonous conditions compared with the homogenous nutrient-rich treatment C1 and homogenous nutrient-zero treatment C2 ($p \leq 0.05$), respectively. The results demonstrated that due to mutual translocation of resources among connected ramets in all heterogeneous treatments increasing heterogeneous patch scale and distribution order in the habitats did not always affect the performance of clonal growth of *Z. japonica* but the interactive effect of nutrient distribution and patch scale significantly affect the growth of *Z. japonica*.

Key words: Clonal integration, Physiological integration, Heterogeneous environment, Homogenous environment, Patch scale, Zoysia japonica.

Introduction

The effect of soil nutrients on the performance of clonal plants through clonal integration under natural and controlled environments at different patch scales remains the subject of debate for more than three decades (Hutchings & Wijesinghe, 1997; Li *et al.,* 2005; Roiloa *et al.,* 2013). Soil nutrient resources are normally unevenly distributed in natural habitats at different scales, and thus, the nutrient resource patterns in the habitats may inconsistently affect the eco-physiological performances of different clonal plants (Alpert & Mooney, 1986; Hutchings & Wijesinghe, 1997a; Liao *et al.,* 2003; Guo *et al.,* 2011). Responses of clonal plants to diverse soil nutrient conditions rely on the topography and sequential dispersal of the nutrients, the size of patches selected for ramet placement, and the capacity of nutrient acquirement (Slade & Hutchings, 1987; Hutchings & Price, 1993; Stuefer *et al.,* 1996). Due to distinct characteristic of phenotypic plasticity of clonal plants and species specific behaviour towards external abiotic stress, we still think it is doubtful that patch scale and the distribution order in the habitats always affect the clonal growth.

Connected ramets may experience different micro-environmental conditions according to existing soil nutrient availability. This may result in different parts of the same ramet or different ramets of the same plant expressing different plastic responses to their local conditions (e.g., Evans, 1988; Stuefer *et al.,* 1994; D'Hertefeldt & Jónsdóttir, 1999). Physiological integration may to some extent explain the underlying mechanism on the performance of the clonal plant species under the various habitats. For instance, in the heterogeneous habitats, ramet under the nutrients rich patch may efficiently uptake more resources, and then through clonal integration, some resources may be translocated to the connected ramet growing under nutrients poor patch (e.g., Evans, 1988; Stuefer *et al.,* 1994; D'Hertefeldt & Jónsdóttir, 1999).

A consequence of small-scale heterogeneity articulated within the individual ramet of a clone is very likely to be simultaneously exposed to a variety of conditions. According to the localized conditions, the responses of ramets may significantly be changed due to clonal integration among connected ramets, both quantitatively and qualitatively (Alpert, 1999a; Dong *et al.,* 2015; Zhang *et al.,* 2016). Reactions of clonal plants to habitats include the responses of its ramets or clonal segments to local conditions, and these responses are modified through physiological integration with other ramets exposed to different conditions (Dong *et al.,* 2015). Therefore, the effect of soil nutrients at different heterogeneous scales on the performance of clonal plant species remains debatable, because most of the previous studies have overlooked the coupling factors such as distribution orders and heterogeneous scales of the soil nutrients under heterogonous environment (but see Zhang *et al.,* 2016).

Resource heterogeneity is a general property of nature (Alpert, 1999a; Dong *et al.,* 2015; Zhang *et al.,* 2016), while natural environment sometime may be expected to be homogeneous at small scale, e.g. the plant scale, compared with the large scale, e.g. the community scale (Evans & Whitney, 1992; Stuefer *et*

al., 1996; Dong *et al.,* 2015). Theoretically, clonal integration may not be more beneficial when ramet growing in the environments where nutrients supply is homogeneous (Caraco & Kelly, 1991; Alpert, 1999b). Most of the previous studies have found the contrasting effects of soil nutrients on the performance of clonal plants under heterogeneous environment compared with the homogenous ones (Evans & Whitney, 1992; Alpert, 1996; Dong *et al.,* 2015). However, the frequently reported positive effects of clonal integration on the performance of clonal plants in the heterogeneous environments may also depend on the different uptake capability of the connected ramets of the different species in resource-rich habitats. It might be expected that the patch scales and distribution orders in the heterogeneous and overall resource limited environments may influence the performance of the clonal plant species, because the capability of response and the resource uptake capacity of the clonal ramet may behave differently at different developing stages and at different resource levels in the patches. Recent studies showed that due to the benefits of physiological integration ramets growing under stressful environments are generally performing better than those under non-stressful environments (Song *et al.,* 2013; You *et al.,* 2013; Roiloa *et al.,* 2014; Zhang *et al.,* 2016).

Over viewing the accumulated research results, we found that the effects of patch scale and distribution order in the habitats on the clonal growth were still vague. Here, we ask a question, do patch scale and distribution order (e.g., from poor to rich patch or vice versa) in the habitats always affect the performance of clonal growth? We used typical stoloniferous clonal plant *Z. japonica* as material, through analyzing the performances of *Z. japonica* clones in the heterogeneous environments with different patch sizes (where amount of nutrient supply was same to all heterogeneous patch levels), levels of patch soil nutrients and patch distribution orders in the habitats, comparing with the homogeneous habitats (Fig. 1), aiming to test the hypothesis that heterogeneous patch scale and distribution order may affect the growth of *Z. japonica*, and their interactive effect also affect the performance of biophysical characteristics of *Z. japonica*.

Material and Methods

Species selection: *Zoysia japonica* is a mat-forming perennial C4 stoloniferous grass, which belongs to Poaceae family (subfamily Chloridoideae, tribe Zoysieae). It is widely distributed throughout Japan islands, Korean peninsula and eastern China from south to north and the other Southeast Asian countries (Li *et al.,* 2005). *Z. japonica* grassland has been used traditionally for livestock grazing and common garden in these countries (Shoji, 1976; Li *et al.,* 2005). The species can form a large interweaves in sunny fields or when planted as lawn, with long creeping stolons. Ecologically grass species play an important role in every ecosystem and provides the major cereal crops and most of the grazing for wild and domestic herbivores. Grasslands are projected to constitute about 20% of the world's vegetation (Ito *et al.,* 2003).

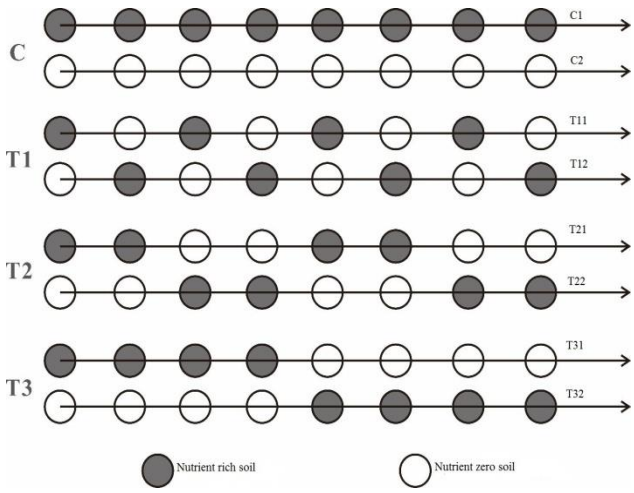

Fig. 1. Conceptual diagram of experimental treatments to the *Zoysia japonica* clones.
C Control group (control), the homogeneous nutrient rich (C1) and nutrient poor treatment (C2); T1: Small scale soil nutrient patch treatment (patch scale is one ramet) (T11: from rich to poor soil nutrient patch; T12: from poor to rich soil nutrient patch); T2: Heterogeneity of soil nutrients doubled than T1 in T2 group medium scale heterogeneous group (similar to T1 T21 starts with nutrient rich tube and T22 starts with nutrient zero tube); T3: Large patches of soil nutrient heterogeneity treatment (big scale heterogeneous group T31 starts with nutrient rich and T32 starts with nutrient poor tube).

Z. japonica possesses a weak shade forbearance and survives in soils varying from infertile sands to clays (Ishida, 1990). It grows better in soils that are slightly alkaline, but *Z. japonica* tolerates acidic soils as well (Ishida, 1990). The hard seed easily sprouts in different soil conditions and the manure of ruminant animals (Ito *et al.,* 2003). The basic morphological component of *Z. japonica* is a 'compound-internode' (Shoji, 1976), which consists of one elongated internode and a couple of shortly compressed internodes (three phytomers). The axillary buds on the proximal and distal short internodes are called as the 'A-' and 'B-tiller', respectively (Shoji, 1976; Ito *et al.,* 2003) Fig. 2.

Experimental design: The experiment started from March 4 to July 4, 2015. All plants used in this experiment were taken from uniform-sized individual ramets severed from a large identical clone cultured in a glass chamber for a few months, and originally taken from an artificial *Zoysia* lawn of the Xinxiang Green Engineering Company, to ensure genetic uniformity among them.

The experiments consist of four types of treatments (Fig. 1), i.e., the homogenous treatments C1 and C2 (C1 was a nutrient-rich treatment with the tubes being filled with sand and nutrient solution with N:P≈7:1), while C2 was a nutrient-zero treatment with the tubes being filled with sand only, and other three heterogeneous treatments with the tubes being filled with sand and nutrient solution with N:P≈7:1 and the patch scales ranging from low to high, i.e., T1, T2 and T3 respectively. In the first heterogeneous treatment T1, every second tube used for receiving ramet root in the experiment was filled with

sand and nutrient solution with N:P≈7:1. T1 treatment consisted of two growing patterns, concretely, T11 started from a nutrient-rich tube internally filled with sand and nutrient solution with N: P≈7:1, and T12 started from a nutrient-zero tube internally filled with sand only (see figure 1). T2 treatment consisted of two growing patterns too, concretely, T21 started from two consecutive nutrient-rich tubes internally filled with sand and nutrient solution with N:P≈7:1, followed by two consecutive nutrient-zero tubes internally filled with sand only, and so on, while T22 had an opposite pattern with T21. The patterns of treatments of T31 and T32 were almost as same as T21 and T22, respectively, with only one difference that the number of consecutive tubes was 4. Each treatment replicated three times. Amount of nutrient supplied in each nutrient-rich tube and that of sand in each tube was same in all heterogeneous treatments (T1, T2 and T3), while the patch scale increased in each treatment. Nutrients applied twice a week to each nutrient rich tube. Every time, 5 ml amount of nutrient solution. Hoagland solution was used for all nutrient rich treatments.

Sampling and measurement: All the plants were harvested in four months, and washed carefully without damaging roots, leaves and stolons. Root morphology of each plant was scanned, then all plants were oven dried at 70° C for 48 hrs, and after wards the numbers of ramets, stolon length, as well as the biomass of the component parts of the plants were measured. Data were analyzed using one way ANOVA to check significant difference between biomass and number of ramets among homogeneous and heterogeneous treatments. Differences between treatments and interaction were analyzed using two-way analysis of variance (two way ANOVA). Independent t-test was used to calculate the significant effects of nutrient treatment and patch scale.

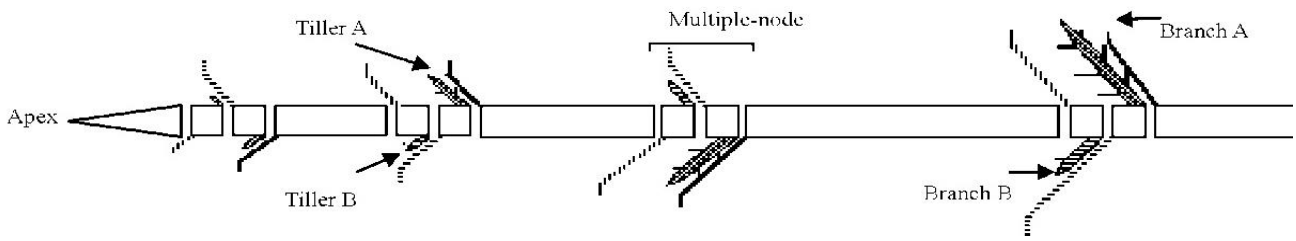

Fig. 2. The diagram of typical morphology of *Zoysia japonica* clone (cited from Dezhi *et al.*, 2006).

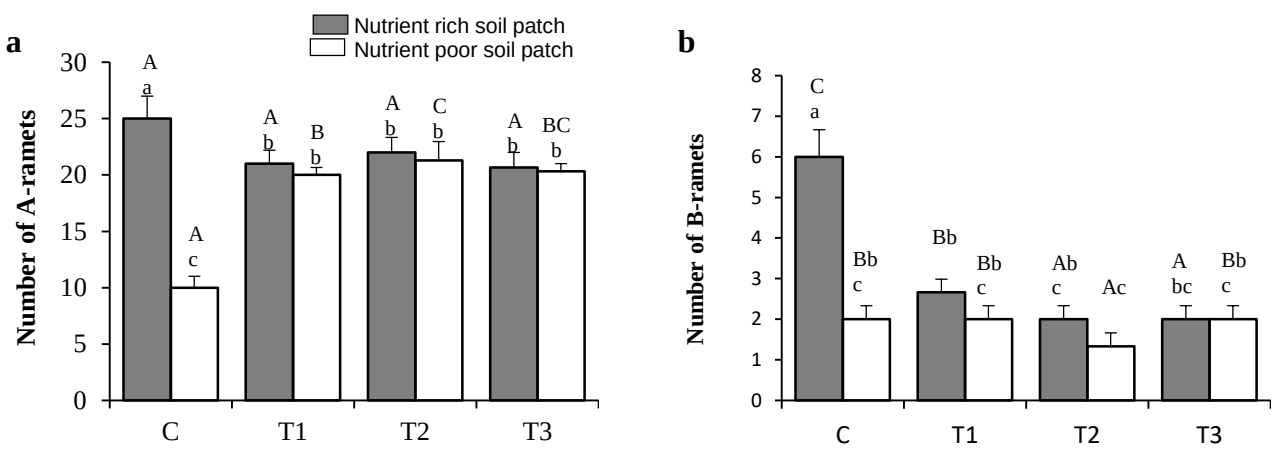

Fig. 3 Soil heterogeneity of nutrient rich patches and barren patches of *Z. japonica* A and B Number of ramets C belongs to control (C1 nutrient rich soil, C2 nutrient poor soil), T1, T2 andT3 are low to high heterogeneous levels

Fig. 3a Number of A- and B-ramets (Fig. 3b) of *Zoysia japonica* at the nutrient rich soil patches and nutrient poor soil patches in the heterogeneous environment.

Bars sharing same small letter means no significant differences within the treatments ($p>0.05$) Bars sharing different small letter means significant difference among treatments ($p<0.05$) One way ANOVA.

Bars sharing same capital letter means no significant difference among nutrient rich or nutrient poor treatments (same colour bars sharing same capital letter means no significant difference) ($p>0.05$) Bars sharing different capital letter means significant difference among treatments ($p<0.05$) Two way ANOVA.

Results

One way ANOVA results showed that there were no significant differences in terms of ramet growth (Fig. 3), branches, root, stolon and total ramet biomass (Fig. 4) and root growth (length, surface area, volume and average root diameter (Fig. 5) under different types of heterogeneous environments with different heterogeneous scales (Table S1). Overall growth of*Z. japonica* in the homogeneous nutrient-zero treatment C2 was significantly lower compared with the homogeneous nutrient-rich treatment C1 and all heterogeneous patch treatments T1, T2 and T3

(Figs. 3, 4, 5). However, the growth of *Z. japonica* in all heterogeneous treatments was significantly lower compared with the nutrient-rich homogeneous treatments C1 (Figs. 3, 4, 5). Although using two way analysis of variance, heterogeneity, nutrient distribution and their interaction significantly affected the growth of *Z. japonica*. Number of A-ramets did not significantly differ in all heterogeneous treatments (Table 1).

In all heterogeneous treatments with different patch scales (T1, T2 and T3), no significant difference was found in terms of number of A- ramets (Fig. 3a, 3b, $p \geq 0.05$), A- branch biomass (Fig. 4a), root biomass, stolon biomass (Fig. 4c, 4d), total ramet biomass (Fig. 4e), total biomass (4f) root length (RL), root surface area (RSA) and root volume (RV) (Fig. 5a, b, c, respectively). But nutrient rich and nutrient poor patches differed among all treatments C, T1, T2 and T3 (Table 1). Number of B-ramets differed significantly at T1 and T2 and B-branch biomass only differed at T2 respectively (Fig. 3b, 4b). Only root average diameter (RAD) was significantly higher in nutrient poor homogeneous treatment C2 and no significant difference was found among homogeneous treatment C1 and all heterogeneous treatments with different patch scales, T1, T2 and T3 (Fig. 5d).

Nutrient patch scale, soil distribution order and their interaction significantly affected the growth of *Z. japonica* in all treatments regardless it started from the nutrient rich or nutrient poor patch (see Tables S1 and S2). Overall growth of *Z. japonica* was significantly low in nutrient poor homogeneous treatment (C2) as compared to all treatments. Homogeneous nutrient rich treatment C1 had significantly higher number of A and B-ramets, A and B branch biomass, root biomass, stolon biomass, total ramet biomass, root length, root surface area and root volume compared with all heterogeneous treatments T1, T2 and T3 (Figs. 3, 4, 5, $p \leq 0.05$). Homogeneous zero nutrient treatment C2 had significantly lower number of A-ramets than that in the heterogeneous treatment (Fig. 3a, $p \leq 0.05$). Contrary to the number of A-ramet, the number of B-ramets was not significantly different from all heterogeneous treatments except for T2, which had lower number of B-ramets (Fig. 2b).

Discussion

Clonal integration between connected ramets adjust the growth of clonal plant at individual ramet and genet level, enables the plants to deal with intricate environmental conditions. In the heterogeneous habitats, in order to maintain the survival or the growth of the ramets in the nutrient-poor patches, overall growth may be sacrificed. This might be the reason overall growth of *Z. japonica* decreased in all heterogeneous treatments compared with nutrient rich homogeneous treatment C1 (Alpert, 1999b; Birch & Hutchings, 1994; Li *et al.*, 2005). Simultaneously, due to vigilant nutrient translocation between connected ramets overall growth of *Z. japonica* did not compromise in nutrient poor patches in (T1, T2 and T3). Growth characteristics of *Z. japonica* were found to be significantly affected by different nutrient distribution in soil. Compared to all heterogeneous nutrient conditions (T1, T2 and T3), the growth characteristics were relatively higher in nutrient rich homogeneous conditions (C1).

Cost of the contributor and the benefit of the recipient as well as the impact on the overall growth of the whole clone may depend largely on the scales, intensity and contrast of the patch (Friedman & Alpert, 1991; Hutchings & Wijesinghe, 1997a,b; Birch & Hutchings, 1994). Contrary to our results, Hutchings & Wijesinghe, (1997) found that the patch scale of heterogeneous habitats had significant effects on the development and growth of root/shoot ratio of entire *Glechoma hederacea* plants, and significantly higher biomass production occurred in the habitats with bigger patches than that in the habitats with smaller patches. Moreover, Qian *et al.*, (2014) found more biomass production and ramet production of *Buchloe dactyloides* only at nutrient rich soil patches and at biggest nutrient patch. They suggested that morphological changes regarding patch scale may occur at certain level of environmental heterogeneity. Conversely, Luo *et al.*, (2013) found *Buchloe dactyloides* grew more efficiently under small and middle scale patches. These results indicated that phenotypic response and clonal integration may vary according to given conditions and essentially depends on species nature.

From the design of the experiment, it was obvious that the overall nutrient in all heterogeneous treatments was same, but the patch scale was linearly increased from T1, T2 to T3, and the distribution order within T1, T2 and T3 was set in contrast, i.e., from rich to poor, or vice versa. In all heterogeneous treatments with same overall provision of nutrition, although the patch scale changed from low to high, significant differences were found among *Z. japonica* clones in all nutrient distribution treatments C, T1, T2 and T3 (Table 1) in terms of growth and biomass production. Moreover, the soil nutrient distribution order in these treatments reversed pair-wisely, the growth and biomass production of *Z. japonica* clones in all treatments (C, T1, T2 and T3) were significantly differed among nutrient rich and nutrient poor patches (Figs. 3, 4, 5). It might be understandable because under the same overall provision of nutrition in every pair-wise heterogeneous treatments, the repetition of the patches with various scales and the reversed soil nutrient distribution order in the treatments symmetrically compensated the loss early and the gain later or vice versa in (T1, T2 and T3), thus, the growth and biomass production of *Z. japonica* clones in these treatments were significantly lowered as compared to homogeneous nutrient rich treatment C1. Interactive effect of nutrients and heterogeneous scale significantly affected the growth of *Z. japonica*.

Our results showed that ramet specialization to acquire locally abundant resources occurred more effectively only in nutrient rich homogeneous treatment C1 compared with all other treatments (Figs. 3, 4 5), resulting in more total biomass of clonal growth. This finding in *Z. japonica* specified that the advantages of clonal integration are not only significant under heterogeneous conditions, as it has been widely proposed (Alpert & Stuefer, 1997; Huber & Stuefer, 1997; Yu *et al.*, 2001; Liao *et al.*, 2003; Guo *et al.*, 2011; Zhang *et al.*, 2016), but also important in homogeneous nutrient conditions (see also Stuefer, 1998; Dong *et al.*, 2015). Alpert, (1991) anticipated that ramets of *Fragaria chiloensis* only allocated more soil nutrients to clonal growth only above a certain level of soil nitrogen or ramet size. Moreover, Bloom *et al.*, (1985) and Birch &

Hutchings, (1994) suggested that collecting a locally abundant resource was expected to require less effort per unit of resource than gathering a locally scarce one. The effects of low nutrient treatment (C2) on the growth of *Z. japonica* (Figs. 3, 4, and 5) may also be understood in terms of this hypothesis.

Though this is a specially designed experiment, we did not find significant differences in overall biomass production and clonal growth of *Z. japonica* under different heterogeneous conditions, whereas we did find significant in biomass and growth under heterogeneous and different homogeneous nutrient conditions (C1 and C2). In conclusion, our study reveals that the patchy distribution of soil nutrients affects the intra-specific interaction between connected ramets of the stoloniferous *Zoysia japonica*. Both heterogeneous scale and nutrient distribution affected the growth of *Z. japonica*, and as a consequence, growth was reduced in all heterogeneous treatments than nutrient rich homogeneous treatment C1. This study reveals that spatial heterogeneity and nutrient supply have significant effects on *Z. japonica*. Furthers studies are needed to be carried out to find out that vigilant resource translocation may occur at large patch scales in the field or not in *Z. japonica*.

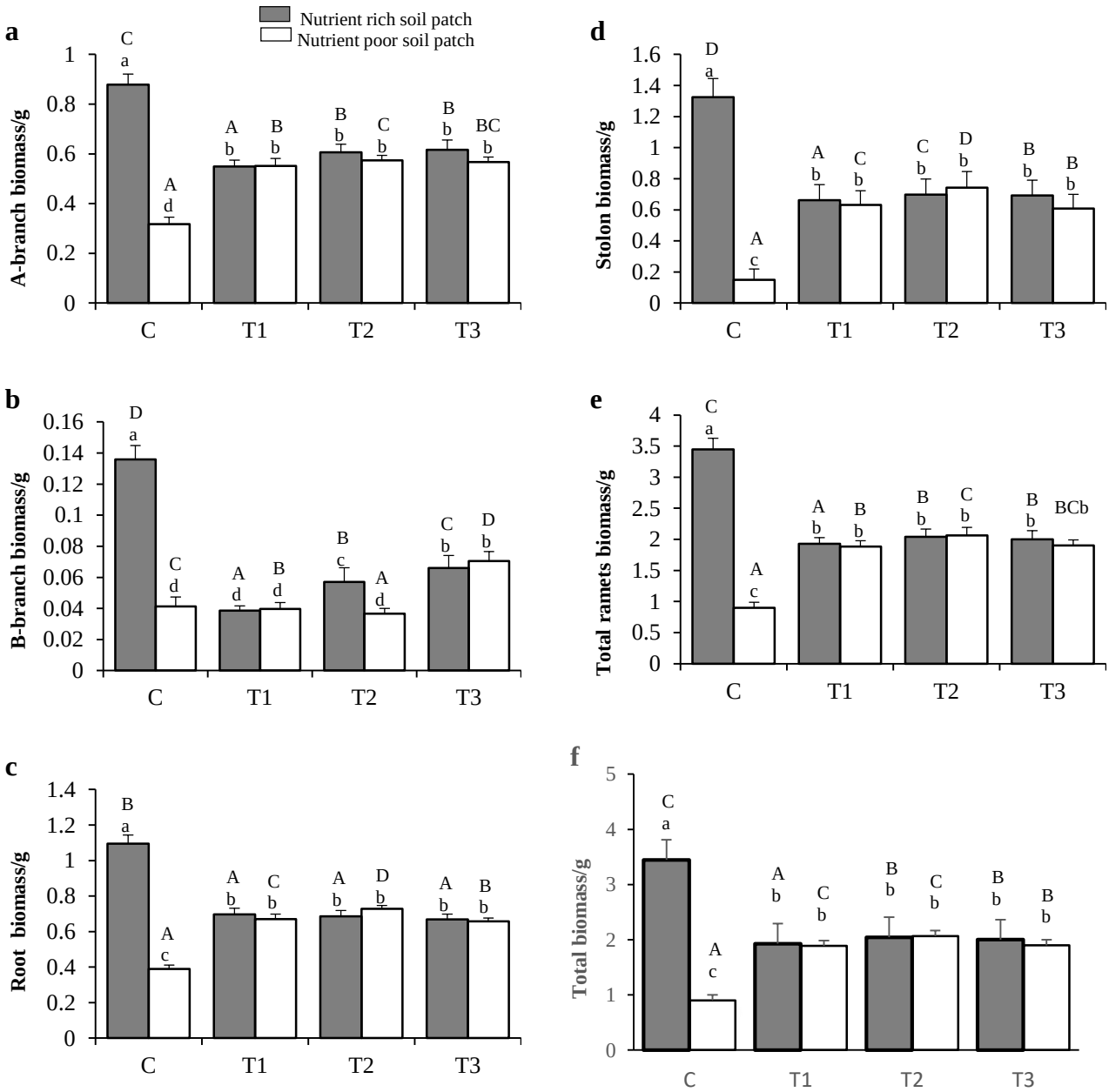

Fig. 4 Soil heterogeneity of nutrient rich patches and barren patches of *Zoysia japonica* A Branch biomass (a), B Branch biomass (b), root biomass (c), stolon biomass (d), total biomass of ramets (e) and total biomass (f). C belongs to control (C1 nutrient rich soil, C2 nutrient poor soil), T1, T2 and T3 are low to high heterogeneous levels

Fig. 4a, A-branch biomass (A), B-branch biomass (4b), root biomass (4c), stolon biomass (4d) and total ramet biomass (4e) of *Zoysia japonica* at the nutrient rich soil patches and nutrient poor soil patches in the heterogeneous environment.

Bars sharing same small letter means no significant differences within the treatments ($p>0.05$) Bars sharing different small letter means significant difference among treatments ($p<0.05$) One way ANOVA.

Bars sharing same capital letter means no significant difference among nutrient rich or nutrient poor treatments (same colour bars sharing same capital letter means no significant difference) ($p>0.05$) Bars sharing different capital letter means significant difference among treatments ($p<0.05$) Two way ANOVA.

Table 1. Two way ANOVA.

Predictor variable effect	Dependent variable	Sum of squares	df	Mean square	F	P
Heterogeneous Treatments	A.-branch biomass	0.008	3	0.003	36.209	0.000
	B-.branch biomass	0.015	3	0.005	1223.359	0.000
	Root biomass	0.010	3	0.003	7.084	0.003
	Total biomass	0.162	3	0.054	31.556	0.000
	Total ramet biomass	0.049	3	0.016	29.438	0.000
	No. of A-branches	34.449	3	11.483	.703	0.564
	No. of B-branches	20.163	3	6.721	1772.539	0.000
	Stolon biomass	0.042	3	0.014	3110.966	0.000
	Root length	156.978	3	52.326	780.203	0.000
	Root surface area	167036.704	3	55678.901	63717.618	0.000
	Root volume	288.818	3	96.273	1656.062	0.000
	Root average diameter	0.081	3	.027	310.569	0.000
Nutrient	A.-branch biomass	0.151	1	0.151	2047.016	0.000
	B-.branch biomass	0.006	1	0.006	1542.724	0.000
	Root biomass	0.186	1	0.186	393.325	0.000
	Total biomass	4.922	1	4.922	2871.848	0.000
	Total ramet biomass	0.808	1	0.808	1446.987	0.000
	No. of A-branches	45.458	1	45.458	2.783	0.115
	No. of B-branches	10.693	1	10.693	2820.224	0.000
	Stolon biomass	0.585	1	0.585	131080.252	0.000
	Root length	660.911	1	660.911	9854.456	0.000
	Root surface area	90460.552	1	90460.552	103520.917	0.000
	Root volume	39.675	1	39.675	682.480	0.000
	Root average diameter	0.006	1	0.006	71.719	0.000
Heterogeneous Treatments* Nutrient	A-.branch biomass	0.324	3	0.108	1464.262	0.000
	B-.branch biomass	0.010	3	0.003	807.441	0.000
	Root biomass	0.570	3	0.190	402.338	0.000
	Total biomass	5.753	3	1.918	1118.890	0.000
	Total ramet biomass	1.985	3	0.662	1185.553	0.000
	No. of A-branches	336.192	3	112.064	6.860	.003
	No. of B-branches	14.642	3	4.881	1287.187	.000
	Stolon biomass	1.502	3	0.501	112304.943	.000
	Root length	647.687	3	215.896	3219.093	.000
	Root surface area	225181.921	3	75060.640	85897.622	.000
	Root volume	116.300	3	38.767	666.856	.000
	Root average diameter	0.009	3	0.003	32.861	.000

Table S1. Of Two-way ANOVA.

Variable	C1 (nutrient rich)	T1	T2	T3	F	P
A-branch biomass	0.87C	0.54A	0.6B	0.62B	687.66	<0.001
B-branch biomass	0.14D	0.02A	0.06B	0.07C	992.73	<0.001
Root biomass	1.06B	0.69A	0.68A	0.66A	128.02	<0.001
Total ramet biomass	3.3C	1.8A	1.98B	2.0B	448.59	<0001
Total biomass	3.41C	1.94A	2.23B	2.28B	844.68	<0.001
Stolon biomass	1.33 D	0.66A	0.7C	0.69B	138339.2	<0.001
No. of A-branches	25A	20.67A	16.03A	20.55A	1.24	0.3587
No. of B-branches	6.04C	2.62B	2.03A	2.0A	2546	<0.001
Root length	42.38D	20.2C	23.24A	23.82B	5162.41	<0.001
Root surface area	803.58D	392.06C	388.06B	381.73A	185479.2	<0.001
Root volume	21.66C	9.36B	8.22A	8.31A	2016.65	<0.001
Root average diameter	0.67C	0.61B	0.59AB	0.56A	47.49	<0.001
Variable	**C2 (nutrient poor)**	**T1**	**T2**	**T3**	**F**	**P**
A-branch biomass	0.31A	0.55B	0.58C	0.57BC	843.5	<0.001
B-branch biomass	0.04C	0.03B	0.02A	0.07D	1113.63	<0.001
Root biomass	0.36A	0.67C	0.72D	0.65B	1096.35	<0.001
Total ramet biomass	0.7A	1.85B	1.99C	1.77BC	1488.55	<0.001
Total biomass	0.85A	1.89C	1.85C	1.64B	365.86	<0.001
Stolon biomass	0.15A	0.63C	0.74D	0.61B	30505.1	<0.001
No. of A-branches	9.97A	19.67B	21.27C	20.34BC	637.78	<0.001
No. of B-branches	2.04B	2.03B	1.28A	2.0B	131.69	<0.001
Root length	14.51A	20.7B	21.57C	20.38B	354.01	<0.001
Root surface area	345.26A	383.92D	377.82C	368.03B	825.44	<0.001
Root volume	11.47C	9.22B	8.54A	8.03A	131.1	<0.001
Root average diameter	0.77C	0.62B	0.59A	0.59A	747.67	<0.001

Table S2. Independent t test.

Homogeneous treatments	Variable	N.R	N.P	Mean (1)	Mean (2)	T	df	p-value
1	A-branch biomass	1.00	2.00	0.87	0.31	66.94	4	<0.0001
1	B-branch biomass	1.00	2.00	0.14	0.04	31.82	4	<0.0001
1	Root biomass	1.00	2.00	1.06	0.36	21.76	2	0.0021
1	Total biomass	1.00	2.00	3.41	0.85	77.23	4	<0.0001
1	Total ramet biomass	1.00	2.00	2.07	0.7	39.66	4	<0.0001
1	No. of A-branches	1.00	2.00	25	9.97	125.08	4	<0.0001
1	No. of B-branches	1.00	2.00	6.04	2.04	78.21	4	<0.0001
1	Stolon biomass	1.00	2.00	1.33	0.15	531.31	4	<0.0001
1	Root length	1.00	2.00	42.38	14.51	143.82	4	<0.0001
1	Root surface area	1.00	2.00	803.58	345.26	501.61	4	<0.0001
1	Root volume	1.00	2.00	21.66	11.47	52.94	4	<0.0001
1	Root average diameter	1.00	2.00	0.67	0.77	-7.77	4	0.0015
Hetro treatments T1	**Variable**	**N.R**	**N.P**	**Mean (1)**	**Mean(2)**	**T**	**df**	**p-value**
2	A-branch biomass	1.00	2.00	0.54	0.55	-0.83	4	0.4507
2	B-branch biomass	1.00	2.00	0.02	0.03	-7.31	4	0.0019
2	Root biomass	1.00	2.00	0.69	0.67	2.18	4	0.0945
2	Total biomass	1.00	2.00	1.94	1.89	1.24	2	0.3401
2	Total ramet biomass	1.00	2.00	1.26	1.25	0.7	4	0.5245
2	No. of A-branches	1.00	2.00	20.67	19.67	2.12	4	0.1012
2	No. of B-branches	1.00	2.00	2.62	2.03	8.43	4	0.0011
2	Stolon biomass	1.00	2.00	0.66	0.63	219.09	4	<0.0001
2	Root length	1.00	2.00	29.69	20.7	59.53	4	<0.0001
2	Root surface area	1.00	2.00	392.06	383.92	10.13	4	0.0005
2	Root volume	1.00	2.00	9.36	9.22	0.6	4	0.5783
2	Root average diameter	1.00	2.00	0.61	0.62	-1.37	4	0.2425
Hetro treatments T2	**Variable**	**N.R**	**N.P**	**Mean (1)**	**Mean (2)**	**T**	**df**	**p-value**
3	A-branch biomass	1.00	2.00	0.6	0.58	3.82	4	0.0188
3	B-branch biomass	1.00	2.00	0.06	0.02	61.43	4	<0.0001
3	Root biomass	1.00	2.00	0.68	0.72	-5.45	4	0.0055
3	Total biomass	1.00	2.00	2.23	1.85	11.48	4	0.0003
3	Total ramet biomass	1.00	2.00	1.34	1.31	4.2	4	0.0137
3	No. of A-branches	1.00	2.00	16.03	21.27	-0.8	2	0.5094
3	No. of-B branches	1.00	2.00	2.03	1.28	14.68	4	0.0001
3	Stolon biomass	1.00	2.00	0.7	0.74	-46.2	4	<0.0001
3	Root length	1.00	2.00	23.24	21.57	13	4	0.0002
3	Root surface area	1.00	2.00	388.81	377.82	12.58	4	0.0002
3	Root volume	1.00	2.00	8.22	8.54	-1.72	4	0.16
3	Root average diameter	1.00	2.00	0.59	0.59	-0.23	4	0.8313
Hetro treatments T3	**Variable**	**N.R**	**N.P**	**Mean (1)**	**Mean (2)**	**T**	**df**	**p-value**
4	A-branch biomass	1.00	2.00	0.62	0.57	8.54	4	0.001
4	B-branch biomass	1.00	2.00	0.07	0.07	-3.68	4	0.0212
4	Root biomass	1.00	2.00	0.66	0.65	1.42	4	0.229
4	Total biomass	1.00	2.00	2.28	1.64	18.86	4	<0.0001
4	Total ramet biomass	1.00	2.00	1.35	1.29	7.34	4	0.0018
4	No. of A-branches	1.00	2.00	20.55	20.34	0.59	4	0.5874
4	Stolon biomass	1.00	2.00	0.69	0.61	34.26	4	<0.0001
4	Root length	1.00	2.00	23.82	20.38	10.77	4	0.0004
4	Root surface area	1.00	2.00	381.73	368.03	46.62	4	<0.0001
4	Root volume	1.00	2.00	8.31	8.03	1.58	4	0.1895
4	Root average diameter	1.00	2.00	0.56	0.59	-5.23	4	0.0064

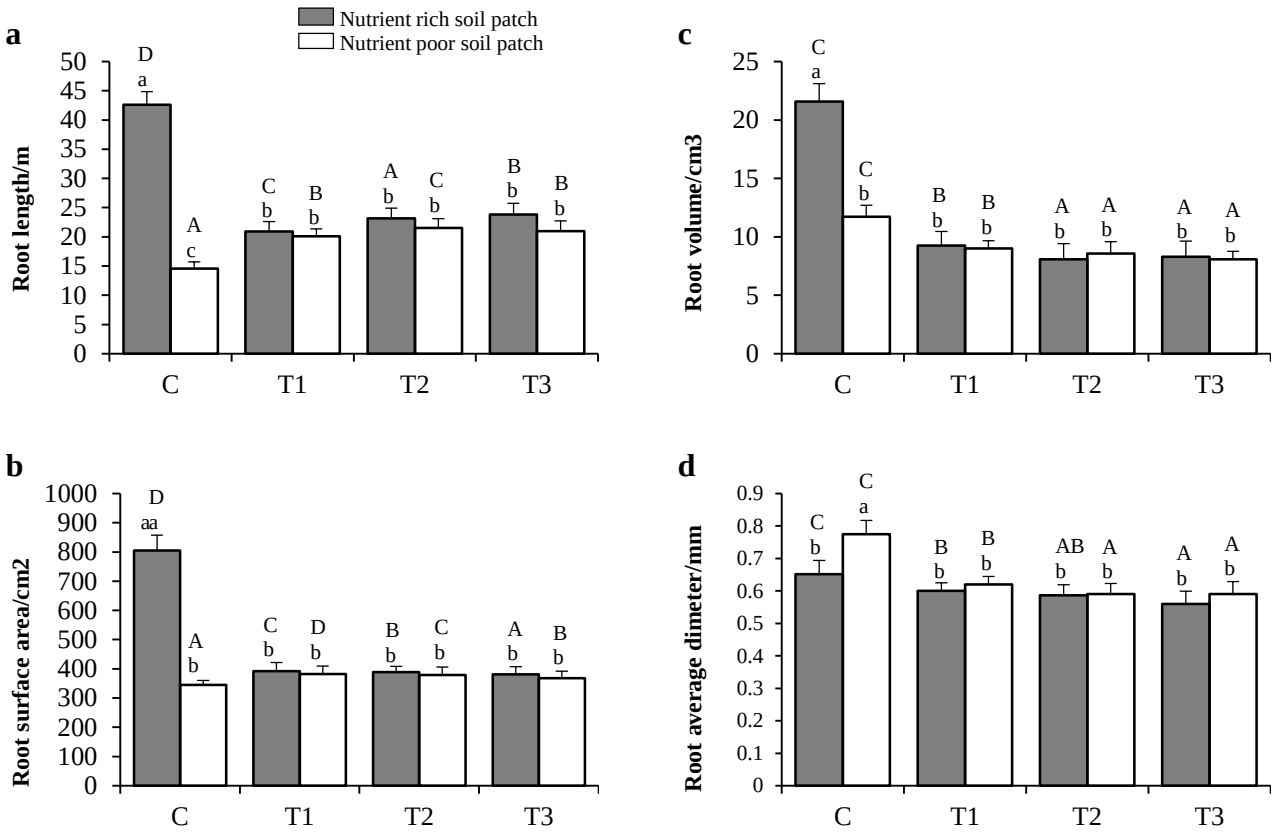

Fig. 5 Soil nutrient heterogeneity of nutrient rich patches and barren patches of clonal ramets

Fig. 5 Root length (5a), root surface area (5b), root volume (5c) and root average diameter (5d) of *Zoysia japonica* ramets at the nutrient rich soil patches and nutrient poor soil patches in the heterogeneous environment. C belongs to control (C1 nutrient rich soil, C2 nutrient poor soil), T1, T2 and T3 are low to high heterogeneous levels

Bars sharing same small letter means no significant differences within the treatments ($p>0.05$) Bars sharing different small letter means significant difference among treatments ($p<0.05$) One way ANOVA.

Bars sharing same capital letter means no significant difference among nutrient rich or nutrient poor treatments (same colour bars sharing same capital letter means no significant difference) ($P>0.05$) Bars sharing different capital letter means significant difference among treatments ($p<0.05$) Two way ANOVA.

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References

Alpert, P. and H.A. Mooney. 1986. Resource sharing among ramets in the clonal herb, *Fragaria chiloensis*. *Oecologia.*, 70: 227-233. doi: 10.1007/BF00379244

Alpert, P. and J.E. Stuefer. 1997. Division of labour in clonal plants. *Ecol. Evol. clon. Pla.*, 137-154.

Alpert, P., 1991. Nitrogen sharing among ramets increases clonal growth in *Fragaria chiloensis*. *Ecology*., 72: 69-80.

Alpert, P., 1996. Nutrient Sharing in Natural Clonal Fragments of *Fragaria Chiloensis*. *J. Ecol.*, 84: 395-406. doi: 10. 2307/2261201

Alpert, P., 1999a. Clonal integration in *Fragaria chiloensis* differs between populations: Ramets from grassland are selfish. *Oecologia*., 120: 69-76. doi: 10.1007/s004420050834

Alpert, P., 1999b. Effects of clonal integration on plant plasticity in *Fragaria chiloensis*. *Plant Ecol.*, 141: 99-106.

Birch, C.P.D. and M.J. Hutchings. 1994. Exploitation of Patchily Distributed Soil Resources by the Clonal Herb *Glechoma-hederacea*. *J. Ecol.*, 82: 653-664. doi: 10.2307/2261272

Bloom, A.J., F.S. Chapin and A.H. Mooney. 1985. Resource limitation in plants- An economic analogy. *Annu. Rev. Ecol. Syst.*, 16: 363-392. doi: 10.1146/annurev.ecolsys.16.1.363

Caraco, T. and C.C.K. Kelly. 1991. On the Adaptive Value of Physiological Integration in Clonal Plants. *Ecology* 72: 81. doi: 10.2307/1938904

D'Hertefeldt, T. and I.S. Jónsdóttir. 1999. Extensive physiological integration in intact clonal systems of *Carex arenaria*. *J. Ecol.*, 87: 258-264. doi: 10.1046/j.1365-2745.1999.00345.x

Dong, B.C., P. Alpert, Q. Zhang and F.H. Yu. 2015. Clonal integration in homogeneous environments increases performance of *Alternanthera philoxeroides*. *Oecologia.*, 179: 393-403. doi: 10.1007/s00442-015-3338-y

Evans, J.P. 1988. Nitrogen translocation in a clonal dune perennial, *Hydrocotyle bonariensis*. *Oecologia.*, 77: 64-68. doi: 10.1007/BF00380926

Evans, J.P., and S. Whitney. 1992. Clonal integration across a salt gradient by a non-halophyte, *Hydrocotyle bonariensis* (Apiaceae). *Am. J. Bot.*, 79: 1344-1347. doi: 10.2307/2445132

Friedman and Alpert. 1991. Reciprocal transport between ramets increases growth of *Fragaria chiloensis* when light and nitrogen occur in separate patches but only if patches are rich. *Oecologia.*,86:76-80.

Guo, W., Y.B. Song and F.H. Yu. 2011. Heterogeneous light supply affects growth and biomass allocation of the understory fern *diplopterygium glaucum* at high patch contrast. *PLoS One.*, doi: 10.1371/journal.pone.0027998

Huber, H. and J.F. Stuefer. 1997. Shade-induced changes in the branching pattern of a stoloniferous herb: Functional response or allometric effect? *Oecologia.*, 110: 478-486. doi: 10.1007/s004420050183

Hutchings, M.J. and A.C.P. Price. 1993. Does physiological integration enable clonal herbs to integrate the effects of environmental heterogeneity? *Pla. Species Biol.*, 8: 95-105.

Hutchings, M.J. and D.K. Wijesinghe. 1997a. Patchy habitats, division of labour and growth dividends in clonal plants. *Trends Ecol. Evol.*, 12: 390-394. doi: 10.1016/S0169-5347(97)87382-X

Hutchings, M.J. and D.K. Wijesinghe. 1997b. the effects of spatial scale of environmental heterogeneity on the growth of a clonal plant: an experimental study with *Glechoma hederacea*. *J. Ecol.*, 85: 17-28. doi: 10.2307/2960624.

Ishida, R. 1990. General remarks on the research works of Japanese lawn grass (*Zoysia japonica* Steud.) and Zoysiatype grasslands in Japan. *J. Japanese Soc. Grassl., Sci*., 36: 210-217.

Ito, M., M. Kodama and T. Okajima. 2003. Regularity in developmental patterns of stolons and tillers of *Zoysia japonica Steud*. Plants growing under a spaced-plant condition. *Grassl. Sci.*, 49:438-43.

Li, D., M. Ito and T. Okajima. 2004. Effects of soil nutrient heterogeneity on the growth of plants Soil nutrient heterogeneity and Zoysia plant growth under the various distributions and levels of nutrients in *Zoysia Japonica* Steud. *Japanese Soc. Grassl. Sci.*, doi: 0.1111/j.1744-6961.2004.00007.x

Liao, M., F. Yu, M. Song, S. Zhang, J. Zhang and M. Dong. 2003. Plasticity in R/S ratio, morphology and fitness-related traits in response to reciprocal patchiness of light and nutrients in the stoloniferous herb, *Glechoma longituba* L. *Act. Oecologica.*, 24: 231-239. doi: 10.1016/j.actao.2003.07.001

Luo, D., Y.Q. Qian, L. Han, J.X. Liu and Z.Y. Sun. 2013. Phenotypic Responses of a Stoloniferous Clonal Plant *Buchloe dactyloides* to Scale-Dependent Nutrient Heterogeneity. *PLoS One.*, 8: 1-8. doi: 10.1371/journal.pone.0067396

Qian, Y.Q., D. Luo, G. Gong, L. Han, G.S. Ju and Z.Y. Sun. 2014. Effects of spatial scale of soil heterogeneity on the growth of a clonal plant producing both spreading and clumping ramets. *J. Plant Growth Regul.*, 33: 214-221. doi: 10.1007/s00344-013-9365-1

Roiloa, S.R., P. Sánchez-Rodríguez and R. Retuerto. 2014. Heterogeneous distribution of soil nutrients increase intra-specific competition in the clonal plant *Glechoma hederacea*. *Plant Ecol.*, 215: 863-873. doi: 10.1007/s11258-014-0338-7

Roiloa, S.R., S.R. Echeverría, H. Freitas and R. Retuerto. 2013. Developmentally-programmed division of labour in the clonal invader *Carpobrotus edulis.Biol. Invasions*., 15: 1895-1905. doi: 10.1007/s10530-013-0417-z

Shoji, S. 1976. Development and growth of the stolon of *Z. Japonica Steud*. Ecological studies on the *Zoysia* type grassland (4): 27: 49-59.

Slade and M.J. Hutchings. 1987. Clonal integration and plasticity in foraging behaviour in *Glechoma hederacea*. *J. Ecol.*, 75: 1023-1036. doi: 10.2307/2260311

Song, Y., F.H. Yu, L.H. Keser, W. Dawson, M. Fischer, M. Dong and M. van-Kleunen. 2013. United we stand, divided we fall: A meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia.*, 171: 317-327. doi: 10.1007/s00442-012-2430-9

Stuefer, J.F. 1998. Two types of division of labour in clonal plants: benefits, costs and constraints. *Perspect Plant Ecol. Evol. Syst.*, 1: 47-60. doi: 10.1078/1433-8319-00051

Stuefer, J.F. and E. Biology. 1996. Potential and limitations of current concepts regarding the response of clonal plants to environmental heterogeneity. *Vegetatio*., 127: 55-70. doi: 10.1007/bf00054847

Stuefer, J.F., H. DeKroon, and H. During. 1996. Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. *Funct. Ecol.*, 10: 328-334. doi: 10.2307/2390280

Stuefer, J.F., H.J. During and H. de-Kroon. 1994. High benefits of clonal integration in two stoloniferous species, in response to heterogeneous light environments. *J. Ecol.*, 82: 511-518. doi: 10.2307/2261260

You, W., D. Yu, C. Liu, D. Xie and W. Xiong. 2013. Clonal integration facilitates invasiveness of the alien aquatic plant *Myriophyllum aquaticum L.* under heterogeneous water availability. *Hydrobiologia.*, 718: 27-39. doi: 10.1007/s10750-013-1596-4

Yu, F., Y. Chen and M. Dong. 2001. Clonal integration enhances survival and performance of *Potentilla anserina*, suffering from partial sand burial on Ordos plateau, China. *Evol. Ecol*., 15: 303-318. doi: 10.1023/A:1016032831038

Zhang, H., F.Liu, R. Wang and J. Liu. 2016. Roles of Clonal Integration in both Heterogeneous and Homogeneous Habitats. *Front. Plant Sci.*, 7: 551. doi: 10.3389/fpls.2016.00551.

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