Contrasting Effects of Plant-Soil Feedbacks on Growth and Morphology of Two Clonal Plants

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Abstract

Aim

Soil abiotic and biotic conditions are often spatially variable, challenging plants with a heterogeneous environment consisting of favorable and unfavorable patches of soil. Many stoloniferous clonal plants can escape from unfavorable patches by elongating stolon internodes, but aggregate in favorable ones through shortening stolon internodes. However, whether these plants can use their stolons to respond to plant-soil feedbacks (PSFs) is largely unknown.

Methods

In the conditioning phase, we grew either *Hydrocotyle vulgaris* or *Glechoma longituba* clonal plants separately in mesocosms to condition bulk soil. In the feedback phase, we grew connected mother and daughter ramets of each species in soil inoculated with the unsterilized or sterilized soil conditioned by conspecifics. We grew the plants for 12 weeks and measured the growth of the mother and daughter ramets separately.

Results

The daughter ramets of *H. vulgaris* produced more biomass but shorter stolon internodes when grown in soil with sterilized inocula than with unsterilized inocula. However, no difference was found between the daughter ramets of *G. longituba* grown in soil with unsterilized and sterilized inocula. For both species, no significant difference was found between the mother ramet or between the daughter ramets when the mother ramet was grown in soil with sterilized and unsterilized inocula.

Conclusions

The daughter ramets rather than the mother ramet of *H. vulgaris* experienced negative biotic PSFs. However, PSF had no effects on the daughter or mother ramet of *G. longituba*. Moreover, physiological integration or plasticity in stolon internode lengths cannot help *H. vulgaris* alleviate the negative PSFs.

Introduction

Plants can change properties of the soil where they grow, and these changes can in turn influence the performance of other conspecific or heterospecific plants either positively or negatively (Bever 1994; Ehrenfeld et al. 2005; van der Putten et al. 2013). These interactions, also known as plant-soil feedbacks, can facilitate subsequent plant performance through soil nutrient accumulation (Berendse 1990; Chapman et al. 2006) and symbiotic mutualist development (Klironomos 2002; van der Putten et al. 2016), or inhibit it through soil nutrient depletion (Berendse 1994) and pathogen accumulation (van der Putten et al. 2016). However, the negative feedbacks are more commonly observed in nature (Kulmatiski et al. 2008). A large number of plants can propagate vegetatively by producing connected genetically identical individuals (ramets) via clonal growth, and these clonal plants are predominant in many local

communities (Klimes et al. 1997; Song and Dong 2002; Klimešová et al. 2018). As offspring ramets of clonal plants are commonly produced near their mother ramets, plant-soil feedbacks may affect both the performance of mother and offspring ramets (Carteni et al. 2012; D'Hertefeldt and van der Putten 1998; van der Stoep et al. 2002).

Many clonal plants can produce rhizomes or stolons of strong plasticity in response to environmental variation in terms of their internode length (de Kroon and Hutchings 1995; Xie et al. 2014). Plasticity of stolon and rhizome internode (hereafter also referred to as “internodes” for brevity) length allows clonal plants to have a higher opportunity to encounter resource-rich patches, and consequently a greater ability to adapt to heterogeneous environments (de Kroon and Hutchings 1995; Gao et al. 2012; Roiloa et al. 2014; van Kleunen and Fischer 2001; Xue et al. 2018c; Ye et al. 2006). In general, clonal plants would produce shorter internodes to utilize adequate resources in favorable habitats, while longer ones can help to “escape” from unfavorable patches (Benedek et al. 2017; de Kroon and Schieving 1990; Dong 1993; Si et al. 2020; Oborny and Cain 1997; Ye et al. 2006). This morphological response in clonal plants has been extensively linked to external environmental factors such as nutrient, water and light availabilities (Dong 1993; Hagiwara et al. 2010; Wijesinghe et al. 2001). However, it is still unclear whether plant-soil feedbacks are involved in such growth strategies. For example, if a clonal plant experiences negative plant-soil feedbacks, it may produce longer internodes to facilitate its offspring ramets to escape from the local stressful environment. By contrast, if a clonal plant experiences positive plant-soil feedbacks, it may produce shorter internodes to maximize use of local soil resources.

When different ramets of the same clone are located in patches of contrasting resource quality, ramets growing in resource-rich patches can translocate nutrients, water and carbohydrates to the ramets growing in resource-poor patches via the connection of internodes (Alpert 1991; Herben et al. 1994; Song et al. 2013; Xue et al. 2020; Yu et al. 2004). The ability to translocate resource among interconnected clonal plants, also known as clonal integration, can benefit certain section(s) of clonal plants experiencing various environmental stresses, including submergence (Luo et al. 2014; Xiao et al. 2010), sand burial and wind erosion (Yu et al. 2004, 2008), salinity (Hester et al. 1994; Xiao et al. 2011) and shading (Li et al. 2018; Stuefer et al. 1994; Xu et al. 2010). Similarly, clonal integration may also benefit clonal plants by reducing effects of negative plant-soil feedbacks or by enhancing the effects of positive plant-soil feedbacks (D'Hertefeldt and van der Putten 1998). However, connection between ramets may also increase the risks of spreading harmful soil microbes (e.g. pathogens) rapidly across connected clones (D'Hertefeldt and van der Putten 1998; Vannier et al. 2018), which may suppress the growth of the clonal plants. Therefore, the response of a clonal plant to plant-soil feedbacks may also depend on the positive and negative effects of biotic plant-soil feedbacks of other connected clonal plants via physiological integration.

Here, we conducted an experiment to examine the effects of biotic plant-soil feedbacks in two well-studied clonal plants, *Hydrocotyle vulgaris* and *Glechoma longituba* (Chen et al. 2014; Liu et al. 2017). We first grew either *Hydrocotyle vulgaris* or *Glechoma longituba* clonal plants separately in mesocosms for three months to condition bulk soil. In the feedback phase, we grew connected mother and daughter

ramets of each of the two species in soil inoculated with the unsterilized or sterilized soil conditioned by conspecifics. Specifically, we tested three hypotheses, for both clonal species: (1) daughter or mother ramets grown in bulk soil inoculated with sterilized (conspecific-conditioned) soil will show greater growth compared with those grown in bulk soil inoculated with unsterilized (conspecific-conditioned) soil, due to accumulation of species-specific soil-borne pathogens in the unsterilized soil (i.e. negative conspecific plant-soil feedbacks); (2) daughter or mother ramets grown in bulk soil inoculated with unsterilized soil will grow better when connected to ramets growing in bulk soil inoculated with sterilized than with unsterilized soil, due to clonal integration; (3) daughter or mother ramets grown in bulk soil inoculated with unsterilized soil will show greater internode growth to show escape response from these unsterilized soil patches.

Materials And Methods

The species

Hydrocotyle vulgaris L. (Araliaceae) is a perennial clonal herb that commonly occurs in moist habitats such as bogs, valleys, dune grasslands and moorlands (Dong 1995). It can produce creeping stems with many nodes; each node has the potential to develop into a ramet consisting of a single leaf and adventitious roots (Dong et al. 2015). This species exhibits rapid clonal reproduction and high morphological plasticity (Dong et al. 2015; Dong 1995). *Glechoma longituba* (Nakai) Kupr. (Lamiaceae) is a perennial clonal herb that can produce ramets connected by stolons. Each ramet has two single leaves originating from a stolon node and some adventitious roots (Liao et al. 2003). The plant is widely distributed in grasslands and forests, on roadsides or by creeks (Liao et al. 2003).

Soil conditioning phase

To create bulk soil, we collected soil from a barren hill in Taizhou, Zhejiang Province, China, air-dried and being sieved (2-cm-mesh) and then homogeneously mixed with river sand at a 1:1 volume ratio. The bulk soil was used to fill eight pots (1.5 L), each with 3.5 kg, and grown with plants for soil conditioning. Before filling the pots, we placed a piece of non-woven fiber at the bottom of each pot to avoid soil from running out the pots.

On 28 June 2019, we collected ramets of both *H. vulgaris* and *G. Longituba* in the campus of Taizhou University in Jiaojiang District, Taizhou, Zhejiang Province, China. We planted these ramets individually in cells (6.5 cm × 6.5 cm × 6.0 cm) on the seedling plates filled with sterilized potting soil (Hebei Dewoduo Fertilizer Co., LTD, Hengshui, China). Sufficient water was supplied daily. Ten days after transplantation, we selected 20 similar-sized ramets of each species and planted them into the pots. The remaining cultivated ramets were allowed to propagate vegetatively until the start of treatments in the feedback phase as described below.

For each species, we planted five ramets in each pot (Fig. 1) and grown for a period of 12 weeks to condition the soil. Each plant species had four replicates, making a total of 8 pots. We replaced dead

ramets during the first week of the treatment. All pots were watered daily to promote plant growth.

After 12 weeks, plants from each pot were harvested and large roots were removed from the soil by hand before being homogenized. After which, the soil from each pot was divided into two equal parts. One part of the soil was sterilized by autoclaving at 121 °C for 120 minutes, and the other part was not sterilized. In total, 16 soil samples were created at the end of the conditioning phase (2 species × 2 sterilization treatments × 4 replicates).

Feedback phase

Each of the 16 soil samples from the conditioning phase was homogenized with the sterilized bulk soil (autoclaving at 121 °C for 120 minutes) at a ratio of 1:9 (W:W), resulting in eight soil samples inoculated with the sterilized soils conditioned by either of the two plant species and another eight soil samples inoculated with unsterilized soils conditioned by either of the two plant species. Each of these 16 soil samples were treated as experimental “blocks” and further divided to fill two pots (12 cm in diameter and 10 cm in height) and two polyvinyl chloride (PVC) trays (100 cm long × 15 cm wide × 8 cm high; Fig. 1). Four treatment combinations for each plant species were created using these filled pots and trays containing soil inoculated with sterilized or unsterilized soil conditioned by conspecifics: 2 pot soil inoculum treatments (sterilized, unsterilized) × 2 tray soil inoculum treatments (sterilized, unsterilized). Each of these soil treatment combinations were replicated four times, with each set of pot and trays containing sterilized or unsterilized soil inoculum originating from the same soil sample obtained from the end of the conditioning phase.

For each of the two clonal plants, we selected 16 similar-sized ramets (mother ramets) that had developed a primary stolon with two nodes (potential offspring ramets), from the cultivated ramets reserved for the feedback phase. Each mother ramet was planted in a pot containing either unsterilized or sterilized soils conditioned by conspecifics (Fig. 1), and its connected potential daughter ramets by the stolon were grown in a tray containing either unsterilized or sterilized soils conditioned by conspecifics (Fig. 1). Any further primary stolons produced by the mother ramets in the pots and secondary stolons (i.e., side-branches developing from the primary stolon) produced by ramets in the trays were not allowed to root and left trailing from the pots and trays. The feedback phase was maintained for 12 weeks in the greenhouse. We replaced dead ramets during the first week of the experiment. During this experiment, the daily mean temperature in the greenhouse was 23 °C. All pots were watered every two days.

At the end of the experiment, we harvested leaves, stolons and roots for mother (in pots) and daughter ramets (in trays) separately. We also counted number of ramets and measured total stolon length. Internode length was calculated by using total stolon length divided by the number of nodes along the stolons. All plant materials were oven-dried at 70 °C for at least 48 hr and weighed.

Data analysis

We used a linear mixed-effects model to analyze each of the measured variables (i.e., total mass, root mass, stolon mass, leaf mass, number of ramets and internode length). In this model, inoculum treatment (inoculated with the unsterilized vs. sterilized conspecific-conditioned soil) of the mother ramets, inoculum treatment of the daughter ramets and their interaction were included as fixed factors. Pot identity in the conditioning phase was included as a random factor in order to account for the non-independence of the inoculum. We performed separate linear mixed-effects models for measurements of the daughter ramets, the mother ramets and the whole clone (daughter + mother ramets). In these analyses, significant local effects of inoculum treatment of the daughter (or mother) ramets on the growth of the daughter (or mother) ramets indicate significant plant-soil feedback effects; while significant nonlocal effects of inoculum treatment of the daughter (or mother) ramets on the growth of the mother (or daughter) ramets indicate clonal integration effects (Stuefer et al. 1994). When a significant effect was detected, *Tukey* tests were used for post-hoc comparisons.

All analyses were performed with R (version 3.4.4; <http://www.r-project.org>) in RStudio (version 1.1.423; <http://rstudio.org>). Linear mixed-effects models were fitted with the *nlme* package (version 3.1-128; Pinheiro et al., 2016); Post-hoc comparisons were made using the *glht* function in the *multcomp* package (version 1.4-15). All data were checked graphically for normality and homogeneity of variance. Total mass, root mass, leaf mass and internode length of the whole clone of *H. vulgaris* and total mass of the whole clone of *G. longituba* were log-transformed before analysis.

Results

Effects on growth and morphology of H. vulgaris

In *H. vulgaris*, biomass (total mass, root mass, stolon mass and leaf mass) of the daughter ramets was significantly greater when they were grown in trays containing sterilized conspecific-conditioned soil (LS and SS) than when they were grown in trays containing unsterilized conspecific-conditioned soil (LL and SL; Fig. 2A-D; Table 1A), indicating that there was a significant negative plant-soil feedback effect on the growth of the daughter ramets of *H. vulgaris*. However, the inoculum treatment of the mother ramet did not influence the growth of the daughter ramets, indicating that there was no integration effect on the daughter ramets. Internode length of the daughter ramets was also greater when they were grown in trays containing sterilized conspecific-conditioned soil (LS and SS) than when they were grown in trays containing unsterilized conspecific-conditioned soil (LL and SL; Fig. 2F; Table 1A), indicating that daughter ramets of *H. vulgaris* did not show escape strategy in response to the negative plant-soil feedbacks. There was no treatment effect on the ramet number of the daughter ramets of *H. vulgaris* (Fig. 2E; Table 1A).

The inoculum treatment of the mother ramet or daughter ramets did not influence the growth or morphology of the mother ramet of *H. vulgaris* (Fig. 2; Table 1B), indicating that there was no significant effect of plant-soil feedback or integration on the mother ramet.

The growth and morphology of the whole-clone showed a similar pattern as those of its daughter ramets, except for a significant interactive effect on root mass of *H. vulgaris* (Fig. 2; Table 1).

Effects on growth and morphology of G. longituba

In *G. longituba*, the inoculum treatment of the daughter or mother ramets did not influence the growth measures (total mass, root mass, stolon mass, leaf mass and number of ramets) of the daughter ramets (Fig. 3A-E; Table 2A), indicating that there was no significant effect of plant-soil feedback or integration on the daughter ramets of *G. longituba*. However, internode length of the daughter ramets of *G. longituba* was overall greater when the mother ramet was grown in pots containing unsterilized conspecific-conditioned soil (LL and LS) than when it was grown in pots containing sterilized conspecific-conditioned soil (SL and SS; Fig. 3F; Table 2B), indicating a significant integration effect on the morphology of the daughter ramets.

There was no significant treatment effect on the growth or morphology of the mother ramets of *G. longituba* (Fig. 3; Table 2B).

Total mass, root mass and internode length of the whole clone of *G. longituba* were significantly greater when the mother ramet was grown in pots containing unsterilized conspecific-conditioned soil (LL and LS) than when it was grown in pots containing sterilized conspecific-conditioned soil (SL and SS; Fig. 3A, B and F; Table 2C). Stolon mass and leaf mass of the whole clone of *G. longituba* was significantly greater when the daughter ramets were grown in trays containing sterilized conspecific-conditioned soil (LS and SS) than when they were grown in trays containing unsterilized conspecific-conditioned soil (LL and SL; Fig. 2C-D; Table 2C). Ramet number of the whole clone was not influenced by the inoculum treatments of the mother or daughter ramets (Fig. 2E; Table 2C).

Discussion

Our results showed that the daughter ramets rather than the mother ramet of *H. vulgaris* experienced negative biotic plant-soil feedbacks, but no biotic plant-soil feedbacks were found for either the daughter or mother ramets of *G. longituba*. Moreover, there was no evidence of facilitation by the *H. vulgaris* mother ramets grown in sterilized conspecific-conditioned soil to their connected daughter ramets suffered from negative biotic plant-soil feedbacks. More importantly, the daughter ramets of *H. vulgaris* did not show "escape" responses in terms of internode length from the negative biotic plant-soil feedbacks. These results indicated that biotic plant-soil feedbacks not only differed among different species, but also differed in physically-connected ramets of the same clone. However, physiological integration and stolon plasticity may not be involved in the response of the two clonal plants to the biotic plant-soil feedbacks as what they usually do in response to abiotic stresses (Gruntman et al. 2017; Roiloa et al. 2010; Yu et al. 2004).

Biotic plant-soil feedbacks vary greatly among different plant species, as well as among different genotypes of the same species (Klironomos 2002; Wagg et al. 2015; Xue et al. 2018a). Here, our results

further revealed that biotic plant-soil feedbacks may also differ between the connected ramets of the same clone in *H. vulgaris*. These differences may have important implications for the thriving of the clonal plants in nature as this variation may “blur” the negative feedbacks on the whole clone (Hart et al. 2016).

Although interconnected, the daughter ramets were suppressed by the negative biotic plant-soil feedbacks, but the mother ramet was not. This result was contrast to a previous study showing that soil-borne pathogens had greatly influenced the mother ramets of a rhizomatous clonal grass *Carex arenaria*, but the daughter ramets were optimally defended (D'Hertefeldt and van der Putten 1998). This was explained by the transportation of resources (physiological integration) from the mother ramets to support their connected daughter ramets, as reported in also many other studies (Lu et al. 2020; Song et al. 2013; Wang et al. 2017). In the present study, however, we did not observe a significant facilitation of the mother ramet of *H. vulgaris* to its connected daughter ramets. Therefore, our second hypothesis was not supported. We proposed that local plant defense may have played a major role in driving the distinct responses of the mother and daughter ramets to biotic plant-soil feedbacks (Stuefer et al. 2004). The daughter ramets were more vulnerable in facing species-specific soil pathogens, compared to the mother ramet, despite that they had access to more resources in terms of nutrients and spaces.

While *H. vulgaris* daughter ramets were affected by negative biotic plant-soil feedbacks, it did not produce longer stolon internodes. This result thus does not support our hypothesis that clonal plants can elongate their inter-ramet distance to facilitate their escape from negative plant-soil feedbacks. Studies have shown that clonal plants can elongate their internodes under stressful conditions such as low light intensity (de Kroon and Hutchings 1995), low nutrient availability (Thomas and Hay 2008) and drought (Zhao et al. 2008). However, under stressful conditions, stolon internodes can be shortened rather than elongated compared to non-stressful conditions, as plants may switch their development from vegetative growth to flowering (Navas and Garnier 2002; Stuefer et al. 2004; Ye et al. 2006; Yu and Dong 2003). This is likely the case in our study, as we found that stolon internode of *H. vulgaris* was shortened under the negative effects of plant-soil feedbacks. This result indicated that negative plant-soil feedbacks may less far-reaching than stolon length. Therefore, it may still not be a problem for this species to be flourishing in natural circumstances despite that they cannot escape negative plant-soil feedbacks through elongating stolons, because they may also use the seeds to distribute to better conditions.

We conclude that biotic plant-soil feedbacks can negatively influence the growth of the daughter ramets, rather than the mother ramet, consequently reduce the growth of the whole clone. However, we did not find the evidence that physiological integration can facilitate the growth of the daughter ramets suffered from negative feedbacks, and that the daughter ramets cannot escape from such negative feedbacks through elongation of internode length either. These results were largely attributed to the defenses of the daughter and mother ramets against their local species-specific soil pathogens. However, we should notice that these results were only true for *H. vulgaris*, but not for *G. longituba*, and thus a general conclusion requires investigations with a large number of clonal plants. Moreover, we constrained the growth of the daughter and mother ramets in separate containers where the soil could be treated as

homogeneous by the daughter and mother ramets. However, soil abiotic and biotic conditions are often spatially variable in natural circumstance, challenging plants with a heterogeneous environment consisting of more and less favorable patches of soil (Xue et al. 2018b). It is still unknown whether clonal plants can show escape strategies in response to negative plant-soil feedbacks in a heterogeneous soil.

Declarations

Declaration of Competing Interest

None.

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Tables

Table 1 Effects of the inoculum treatment (inoculated with the unsterilized vs. sterilized conspecific-conditioned soil) of the mother and daughter ramets on the growth and morphology of (A) the daughter ramets, (B) the mother ramet and (C) the whole clone (mother plus daughter ramets) of *Hydrocotyle vulgaris*

Effect	DF	Total mass	Root mass	Stolon mass	Leaf mass	No. of ramets	Internode length
<i>(A) Daughter ramets</i>							
Mother inoculum (M)	1,9	0.03	1.35	0.12	0.51	0.20	0.71
Daughter inoculum (D)	1,9	17.32 ^{**}	16.62 ^{**}	12.97 ^{**}	11.18 ^{**}	2.28	23.47 ^{***}
M x D	1,9	<0.01	2.16	0.52	0.92	0.07	0.26
<i>(B) Mother ramets</i>							
Mother inoculum (M)	1,9	1.49	1.83	1.09	1.04	1.04	0.77
Daughter inoculum (D)	1,9	1.56	2.76	1.39	0.27	0.41	1.66
M x D	1,9	2.98	6.98	1.53	2.23	0.25	0.78
<i>(C) Whole clone</i>							
Mother inoculum (M)	1,9	0.22	0.59	0.21	1.06	0.59	0.44
Daughter inoculum (D)	1,9	21.82 ^{**}	92.83 ^{***}	14.51 ^{**}	10.52 [*]	2.01	11.97 ^{**}
M x D	1,9	0.45	33.76 ^{***}	0.08	2.20	0.39	0.03

Numbers are *F*-values of two-way ANOVAs. Superscripts give *P*. ^{***}*P* < 0.001, ^{**}*P* < 0.01 and ^{*}*P* < 0.05. Total mass, root mass, leaf mass and internode length of the whole clone were log-transformed.

Table 2 Effects of the inoculum treatment (inoculated with the unsterilized vs. sterilized conspecific-conditioned soil) of the mother and daughter ramets on the growth and morphology of (A) the daughter ramets, (B) the mother ramets and (C) the whole clone (mother plus daughter ramets) of *Glechoma longituba*

Effect	DF	Total mass	Root mass	Stolon mass	Leaf mass	No. of ramets	Internode length
<i>(A) Daughter ramets</i>							
Mother inoculum (M)	1,9	2.28	1.05	2.15	2.65	2.85	6.18*
Daughter inoculum (D)	1,9	3.86	3.43	2.75	3.97	1.87	1.98
M x D	1,9	1.18	0.95	1.35	0.84	0.06	0.96
<i>(B) Mother ramets</i>							
Mother inoculum (M)	1,9	0.12	0.42	0.23	0.01	0.60	0.01
Daughter inoculum (D)	1,9	1.39	1.42	2.14	0.66	0.60	0.60
M x D	1,9	0.21	0.57	0.07	0.09	0.02	0.01
<i>(C) Whole clone</i>							
Mother inoculum (M)	1,9	1.85**	0.83**	1.99	3.99	0.66	8.14*
Daughter inoculum (D)	1,9	14.92	12.13	6.34*	7.67*	3.97	3.73
M x D	1,9	2.43	3.74	1.99	1.54	0.01	1.36

Numbers are *F*-values of two-way ANOVAs. Superscripts give *P*. ***P* < 0.01 and **P* < 0.05. Total mass of the whole clone was log-transformed.

Figures

I Conditioning phase

II Feedback phase

Figure 1

Schematic representation of the experimental design. The experiment consisted of two phases. In the conditioning phase, the soil in each pot was grown with five ramets of *Hydrocotyle vulgaris* or *Glechoma longituba* for 12 weeks. In the feedback phase, for each species, a mother ramet was grown in a pot (circle) filled with a bulk soil inoculated with either a unsterilized or a sterilized conspecific-conditioned soil collected from the conditioning phase, and its daughter ramets connected by a primary stolon were grown in a tray (rectangle) filled with the bulk soil inoculated with either a unsterilized or sterilized conspecific-conditioned soil collected from the conditioning phase. Treatment codes: LL – both mother and daughter ramets were grown in the bulk soil inoculated with the unsterilized conspecific-conditioned

soil, LS – the mother and daughter ramets were grown in the bulk soil inoculated with the unsterilized and sterilized conspecific-conditioned soil, respectively, SL – the mother and daughter ramets were grown in the bulk soil inoculated with the sterilized and unsterilized conspecific-conditioned soil, respectively, and SS – both mother and daughter ramets were grown in the bulk soil inoculated with the sterilized conspecific-conditioned soil.

Figure 2

Total mass (A), root mass (B), stolon mass (C), leaf mass (D), number of ramets (E) and internode length (F) of the daughter (upper panel) and mother (lower panel) ramets of *Hydrocotyle vulgaris* under the four treatments (N = 4). Mean values (SE) are given. Different letters indicate significant difference among the treatments. Conspecific-conditioned soil inoculation treatments: LL - "mother" and "daughter" ramets grown in soil inoculated with unsterilized conspecific soil, LS - mother with unsterilized and daughter with sterilised inoculum, SL - mother with sterilized and daughter with unsterilized inoculum, and SS - mother and daughter with sterilized inoculum.

Figure 3

Total mass (A), root mass (B), stolon mass (C), leaf mass (D), number of ramets (E) and internode length (F) of the daughter (upper panel) and mother (lower panel) ramets of *Glechoma longituba* under the four treatments (N = 4). Mean values (SE) are given. Different letters indicate significant difference among the treatments. Conspecific-conditioned soil inoculation treatments: LL - "mother" and "daughter" ramets grown in soil inoculated with unsterilized conspecific soil, LS - mother with unsterilized and daughter with sterilised inoculum, SL - mother with sterilized and daughter with unsterilized inoculum, and SS - mother and daughter with sterilized inoculum.