The impact of defense hormones on the interaction between plants and the soil microbial community

Zhang, J.

Chapter 5

The negative effect of soil microorganisms on plant growth only extends to the first weeks

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Abstract

Soil biotic communities can strongly impact plant performance. Many plant species grow worse in live soil than in sterilized soil. So far, most studies on plant-soil-interactions have estimated the effect of the soil microbial community on plant mass after a fixed duration of plant growth. However, these interactions may change over time and several studies have argued that plant-soil interactions are more important for young seedlings than for older plants. In this paper we ask the question: how long-lasting is the effect of the soil microbial community on plant growth and we focus on relative plant growth rates at three stages: early growth (0-21 days), mid growth (22 to 42 days) and late growth (43 to 63 days). This is important as a plant with a reduced relative growth rate early in life, due to negative effects of the soil microbial community, may increase less in biomass for a much longer period, even though the relative growth rates do not differ anymore. We performed two growth experiments with *Jacobaea vulgaris* lasting 49 and 63 days. Plants were grown in sterilized soil or in sterilized soil inoculated with natural dune soil. In both experiments, differences in biomass of plants grown in sterilized soil and inoculated soil (live soil) increased throughout the experiment. Interestingly, linear regression models testing the relationship between *ln* transformed dry weight and time for younger plants and for older plants in sterilized soil and live soil, respectively, showed that the relative growth rate of plants in the sterilized soil was only significantly higher than that of plants in live soil in the first 2-3 weeks. After that period, there was no negative effect of live soil on plant relative growth rate anymore. In a third experiment, we examined the effect of the timing of soil inoculation prior to planting on the relative growth rate of *J. vulgaris* plants with four different timing treatments. Plant biomass was reduced in all inoculated soils compared to the sterilized soil. With increasing time between inoculation and planting, plant biomass decreased. Again, in all inoculated soils the negative effect of the soil microbial community on plant growth disappeared during the first weeks after planting. Overall, our results show that plants grow less well in live soil than in sterilized soil. The negative effects of soil inoculation on plant mass appear to extend over the whole growth period but arise from the negative effects on relative growth rates that occur in the first weeks after planting when plants have only less than 5% of the mass they obtained after 42 days. Our study highlights the

importance of examining relative growth rates rather than final biomass to estimate the effects of soil microbial communities on plants.

Keywords

Dry plant biomass, Growth analysis, *Jacobaea vulgaris,* Live soil, Plant performance, Plant-soil interactions, Pathogenic soil microbial community, Relative growth rate, Sterilized soil

Introduction

Interactions between plants and soil microbial communities are vital in mediating the balance and functioning of terrestrial ecosystems (Bever, 1994; Churchland and Grayston, 2014; Teste et al., 2017; Erktan et al., 2018). The soil microbiome is an important driver of plant performance. Soil microbial species e.g. pathogenic organisms, plant-growth-promoting rhizobacteria (PGPR, such as *Pseudomonas* and *Burkholderia*) and arbuscular mycorrhizal fungi (AMF), play an active role in modifying the development of plants (Johnson et al., 1997; Arora and Mishra, 2016; Artursson et al., 2016; Gil-Martinez et al., 2018). The effect of the soil microbial community on plant growth in laboratory experiments is often negative (Mangan et al., 2010; van de Voorde et al., 2012; Cortois et al., 2016).

One potential explanation for the negative effect of soil microbes on plant performance is that microbes and plants compete for nutrients. Alternatively, pathogens may accumulate in the soil over time, eventually resulting in a negative overall effect on plant performance (Dobson and Crawley, 1994; Wardle et al., 2004; Raaijmakers et al., 2009; Mordecai, 2011; van der Putten et al., 2013; Jacoby et al., 2017). In the previous chapters, we showed in experiments with ample nutrient supply that the negative effect of the soil microbial community on plant growth is mitigated if the plant's defense system is activated by foliar application of salicylic acid. This led us to hypothesize that the negative effect of the soil microbial community on plant growth in our system is due to an overall pathogenic effect of the soil microbial community. Although this effect was consistent, we did not find this effect to increase over several generations of plant growth (Chapter 2). An important question is therefore how long the negative effects of the soil microbial community on plant growth lasts.

So far, most studies on the effect of the soil microbial community on plant growth are conducted in pots (Hodge and Fitter, 2013). In such experiments, the negative effects of any treatment on plant mass often decline after some period of plant growth (typically six to eight weeks) (Bezemer et al., 2018; Dudenhöffer et al., 2018). This is often attributed to restricted root growth due to limitations in pot size, or to a decline in nutrient availability, and therefore considered an artefact of the experimental design

(Smith and Reynolds, 2012; Van de Voorde et al., 2012; Jing et al., 2015). It is also possible, however, that the pathogenic effect of the soil microbial community only last for a short period because (1) only seedlings are susceptible or (2) because over time plants alter the composition of the microbial community in the soil in which they grow so that it becomes less harmful (Bezemer et al., 2018; Dudenhöffer et al., 2018).

Previous studies on plant-soil-interactions typically focus on the effect of the soil microbial community on final plant biomass (van de Voorde et al., 2012; Bezemer et al., 2013; Anacker et al., 2014). It is important to note, however, that the effects of the soil microbial community on plant growth depend on the life stages of the plant (Arrigoni et al., 2018; Bezemer et al., 2018; Dudenhöffer et al., 2018). Seedlings are often highly vulnerable and susceptible to pathogenic microbes in the soil (Packer and Clay, 2000). In contrast, older plants with a more developed root system are typically less vulnerable (Kardol et al., 2013; Bezemer et al., 2018).

Effects on plant growth that occur during early life stages can still affect plant size and plant phenology in late life stages. When plants after some period grow with a similar relative growth rate, differences in absolute plant mass will still continue to increase. In Fig. 1 it is assumed that plants in sterilized soil grow with a constant relative growth rate (red line). Plants in live soil either grow with a constant relative growth rate lower than that of the plants in the live soil (green line) or they first grow with a lower relative growth rate but after an initial period (t_1) their relative growth rate becomes similar to that of plants in the sterilized soil (blue line). In the latter case, although the effect of the soil microbial community only is present until t_1, differences in plant mass still continue to increase (Fig. 1b). Hence, to study the effect of soil microbes on plants, it is important to analyze relative growth rates.

In this study, we used linear regression models and *ln* transformed biomass data from repeated harvests to estimate relative growth rates in sterilized and live soil. We hypothesized that i) relative growth rates of plants are smaller in live soil than in sterilized soils ii) the negative effect on relative growth rates lasts only for a short period during the early plant life stages; and iii) the differences in plant mass between

plants grown in live soils and sterilized soils will continue to increase during the experiment.

We used *Jacobaea vulgaris* to test these hypotheses. *J. vulgaris* is native to The Netherlands. In a former experiment, we found that the plant mass of *J. vulgaris* growing in soil containing a live microbial community was 66% lower than when plants were grown in sterilized soil (Jing et al., Chapter 2). This negative effect of live soil on plant growth is in line with previous findings (e.g. van de Voorde et al., 2012; Kos et al., 2015; Wang et al., 2019). In the present study, to avoid nutrient limitation during the growth of *J. vulgaris*, nutrients were supplied regularly according to estimates of nutrient demand obtained from previous experiments (Steiner, 1980; Joosten et al., 2009). We carried out growth experiments with multiple harvesting points to estimate changes in relative growth rates in live and sterilized soils. Additionally, we grew *J. vulgaris* plants in soil that had been inoculated with live soil at varying time points before planting to manipulate the abundance of the microbial community in the soil. With the latter experiment we aimed to examine how the timing of inoculation into sterilized soil impacts the growth of *J. vulgaris*.

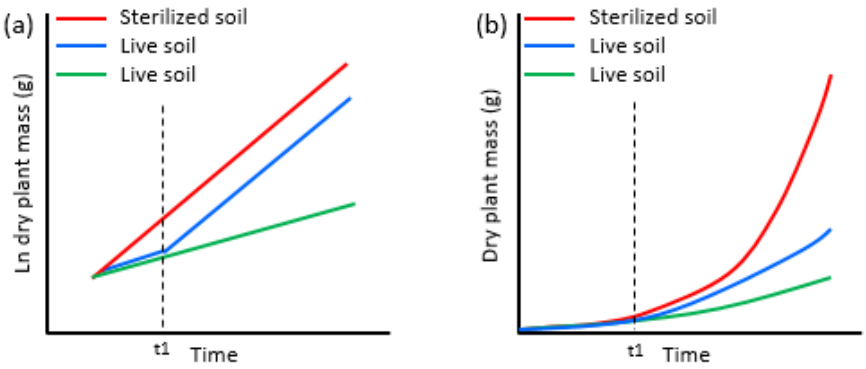

Fig. 1 Conceptual figures showing relative growth rate and plant mass of *J. vulgaris* in both sterilized soil and live soil over time. (a) The relative growth rate in sterilized soil (red line) is higher than that in live soil (green line) (hypothesis 1) and this difference is maintained during the entire plant growth period. The blue line indicates

an initial lower relative growth rate of plants in the live soil but at t_1 these plants obtain an equal relative growth rate as plants in the live soil (hypothesis 2). In Fig. 1a the y-axis denotes *ln* transformed plant mass. In Fig. 1b the y-axis denotes absolute plant mass. Note that even when relative growth rates become equal after an initial difference in relative growth rate in the early stage of life (the red line and blue line in Fig. 1a) the difference in absolute biomass continues to increase after that period (the red line and blue line in Fig. 1b).

Materials and methods

J. vulgaris (common ragwort) was used as plant species. We chose this species because it is a common species in The Netherlands that is strongly affected by plant-soil interactions (van de Voorde et al., 2011; van de Voorde et al., 2012; Bezemer et al., 2013). Seeds and soil were collected from Meijendel, a calcareous sandy dune area north of The Hague, The Netherlands (52°11´N, 4°31´E).

Seeds

Before seed germination, all seeds were shaken for 2 min in 70% ethanol, then washed with sterilized water, put for 12 min in 2% bleach, and finally rinsed four times with sterilized water to avoid influences of seed-borne microbes. The surface-sterilized seeds were then placed in standard Petri dishes containing filter paper, which was moistened with Milli-Q water. Afterwards, all Petri dishes containing seeds were placed in plastic zip-lock bags and stored in a climate room (relative humidity 70%, light 16 h at 20°C, dark 8 h at 20°C) for the duration of germination.

Soil

At Meijendel, the topsoil was collected to a depth of 15 cm after removing the grassland vegetation and the organic layer of the surface. The soil was sieved using a 5 mm sized mesh to remove plant roots and various soil fauna, homogenized with a concrete mixer and then stored into 20-liter plastic bags (Nasco Whirl-Pak Sample Bag). Bags were either sterilized by 35-K Gray gamma-irradiation (Synergy Health Company, Ede, The Netherlands) or kept at 4°C for inoculation. Potting soil

(Slingerland potgrond, Zoeterwoude, The Netherlands) was also sterilized by 35-K Gray gamma-irradiation.

Plant growth

After germination, seedlings were randomly transferred individually to 500 ml pots containing either "sterilized soil" or "live soil". The live soil treatment consisted of a mixture of 87.5% sterilized dune soil, 2.5% sterilized potting soil and 10% live soil. The sterilized soil treatment contained 97.5% of sterilized dune soil and 2.5% of sterilized potting soil. Sterilized potting soil was added to all pots to increase the organic matter content of the soil. Sterilized soil and live soil were kept in bags and left in the climate room for 14 days (relative humidity 70%, light 16h at 20°C, dark 8h at 20°C) to enable the establishment of microbial communities in the inoculated soil before potting. Before filling the pots, the soil in each bag was mixed. After filling, pots were randomly distributed over the climate room. Plants were watered regularly with Milli-Q water and 5 ml Steiner nutrient solution was added per plant on day 7, 10 ml Steiner nutrient solution was added on day 13, and 20 ml Steiner nutrient solution was added on days 19, 28, 37, and 42. The Steiner nutrient solution (Steiner, 1980) was prepared from 7 different stock solutions (106.2 g $Ca(NO_3)_2 \cdot 4H_2O$, 29.3 g KNO_3, 13.6 g KH_2PO_4, 49.2 g $MgSO_4 \cdot 7H_2O$, 25.2 g K_2SO_4 and 2.24 g KOH, 3.29 g Fe-EDTA added to 1 liter demineralized water, and a stock solution with micro elements (a mixed solution of 0.181 g $MnCl_2 \cdot 4H_2O$, 0.286 g H_3BO_3, 0.022 g $ZnSO_4 \cdot 7H_2O$, 0.0078 g $CuSO_4 \cdot 5H_2O$ and 0.0126 g $NaMoO_4 \cdot 2H_2O$ added to 1 liter demineralized water). Ten ml of each stock solution was diluted in 1 liter of demineralized water before use.

The effect of live soil on the growth of *J. vulgaris*

Experiment 1: An experiment to measure the growth of *J. vulgaris* overtime was performed starting with 1-week-old seedlings, two soil treatments and eight harvesting time points over seven weeks. The harvests were on days 0 (1-week-old seedlings), 7, 14, 21, 28, 35, 42 and 49 after planting. Pots were randomly labeled and allocated to the different harvests. Ten replicates were used for each treatment resulting in 2 treatments × 8 harvesting points × 10 replicates = 160 plants. Harvested

plants (shoots and roots) were oven-dried at 60°C for approximately one week and dry mass was determined.

Experiment 2: The growth experiment was repeated using the same soil treatments, but with more harvests during the first 3 weeks. In this experiment, plants were harvested at day 0, 3, 6, 9, 12, 15, 18, 21, 28, 35, 42, 49, 56 and 63 after planting. Ten plants per soil treatment were harvested at each harvesting time point thus resulting in 2 treatments × 14 harvest points × 10 replicates = 280 plants. In this experiment, at each harvest, the plants were gently removed from the pot. Shoots were separated from roots with a pair of scissors just above the root crown, and roots were cleaned with water and then put into aluminum foil. Then, all the harvested plant parts were freeze-dried for approximately one week, and dry mass was determined.

The effect of time of inoculation on the growth of *J. vulgaris*

To examine the effect of the timing of soil inoculation on the relative growth rate of *J. vulgaris* plants, sterilized soils were inoculated at different time points prior to planting the seedlings. In this experiment, 1-week-old seedlings were planted into 500 ml pots containing either “sterilized soil” or four different “live soil” treatments. For these four treatments, a mixture of 10% of live soil was mixed with 90% sterilized soil, and then the mixed soil was kept in the climate room for 0, 1, 2 and 4 weeks (relative humidity 70%, light 16h at 20°C, dark 8h at 20°C) to enable different build-up times for the microbial community in the soil at the time of planting. The live soil treatments were labeled as “live-0”, “live-1”, “live-2” and “live-4” respectively. Seedlings were randomly distributed over the five soil treatments and nine harvests over six weeks. Plants were harvested on days 0 (as seedlings), 4, 8, 12, 16, 20, 28, 35 and 42. Eight replicates were used per treatment combination, resulting in 5 treatments × 9 harvests × 8 replicates totaling 360 plants. Fresh weight was recorded, because leaves were frozen immediately as we intended to quantify the levels of SA in the plant material. However, due time limitations these data have not been collected.

Calculations and statistical analyses

Biomass was plotted against time for plants grown in sterilized and live soil. A student t-test was then performed to test for differences between dry plant mass in sterilized and live soils at each time point. *Ln* transformed biomass was also plotted against time. Plant growth was divided into three stages: early growth (0-21 days), mid growth (22 to 42 days) and late growth (43 to 63 days). For each experiment, a separate line was then fitted through the dry plant mass data for these different periods. Late growth was only measured in experiment 2. Because this division in two time periods is somewhat arbitrary, we backed this analyses up with a sequential backward regression approach for the entire growth period for each experiment. We started this analysis with the two latest harvesting points and then sequentially added the previous data point. In this way we could test for which time periods differences in relative growth rate were significant. For each regression the slope and standard error (SE) of the slope were determined and differences between the slopes for the linear regression models in sterilized and live soil were then tested with a *t*-test in Excel, $t = \frac{\text{Slope1}-\text{Slope2}}{\text{SQRT}\,(SE1^2+SE2^2)}$.

Results

The effect of live soil on the growth of *J. vulgaris*

Experiment 1: Soil inoculation had a strong negative effect on plant dry mass throughout the experiment (Fig. 2a). The difference in plant dry mass between the sterilized and live soil treatments increased during the entire experiment. From day 21 onward, the dry plant mass of *J. vulgaris* in sterilized soil was significantly larger than the dry mass of plants grown in live soil (Fig. 2). For young plants (0-21 days) the relative growth rate (slope in Fig. 2b, c) in sterilized soil was significantly larger than that for live soil while relative growth rates did not differ for mid-aged plants (22-49 days, Fig. 2b, c). This result was backed up by the sequential backward regression that showed that the relative growth rates were not significantly different for the periods between 22 and 49 days (Table S1).

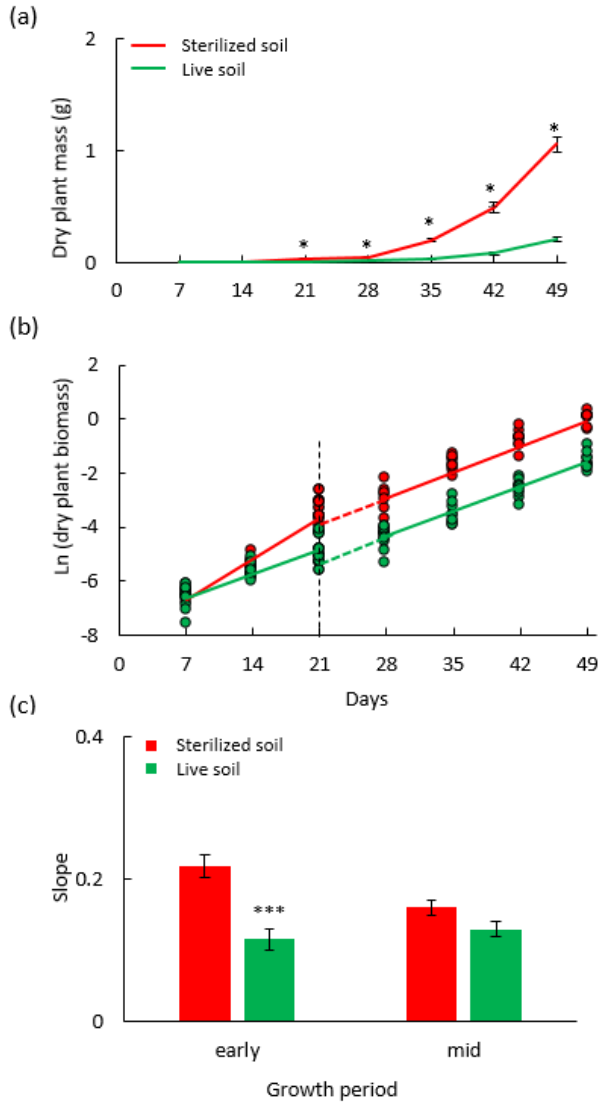

Fig. 2 Experiment 1. (a) Mean (± SE) biomass of *J. vulgaris* in sterilized and live soil over 49 days. For each time point, differences between the biomass of the plants in the two soils were tested for significance with a t-test, * indicates a significant difference ($P < 0.05$). (b) Two linear regression models (early: day 7-21, and mid: day 28-49) of *ln* transformed biomass of *J. vulgaris* in both sterilized and live soil. The extrapolated dashed parts of the lines are based on the linear regression models for day 28-49. (c) Slopes (mean± SE) of the regression lines in (b). Differences between

the slopes for live-soil and control soil were tested for significance with a-test. *** indicates $P < 0.001$.

Experiment 2: The first experiment was repeated with more harvesting points during the first 21 days and an extended growth period. Again, the effect of live soil on plant growth was negative (Fig. 3a). The difference in absolute plant biomass increased until day 56. Young plants (0-21 days) had significantly higher relative growth rates in sterilized soil, mid-aged plants (22-42 days) had similar relative growth rates; while for older plants (49-63 days) the relative growth rates were even higher in live soil (Fig. 3b, c). Backward regression showed that the relative growth rate was higher for the plants in live soil for the period 63-28 days. If younger ages are included in the analysis, differences are no longer significant (Table S2).

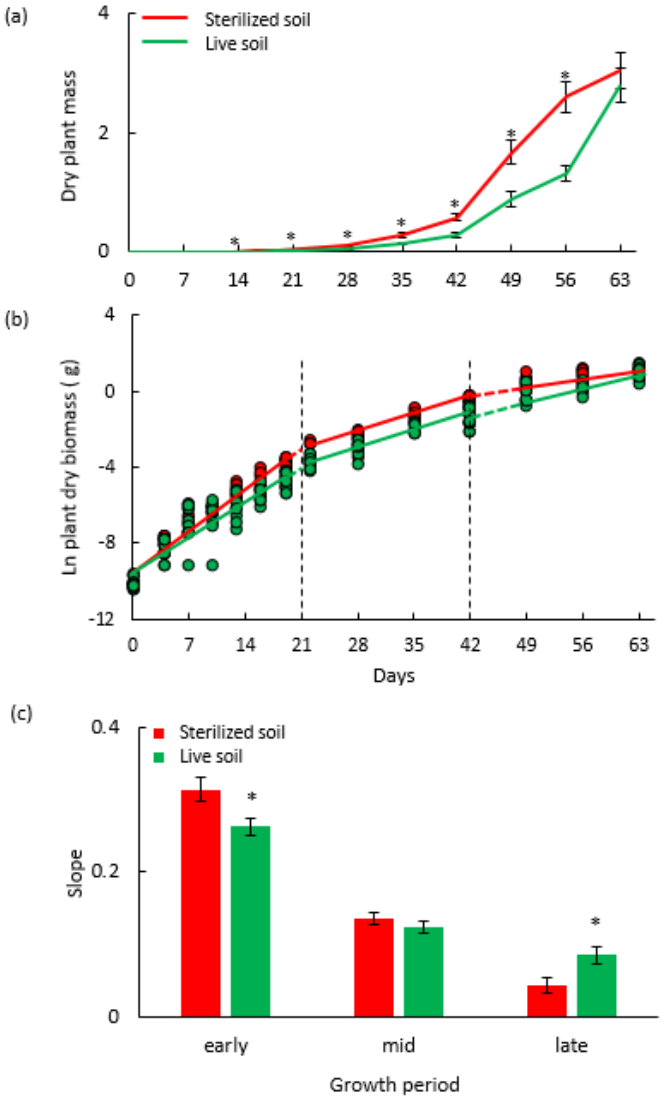

Fig. 3 Experiment 2. (a) Mean (± SE) of dry plant mass of *J. vulgaris* in sterilized and live soil over 63 days. For each time point, differences between the biomass of the plants in the two soils were tested for significance with a t-test, * indicates a significant difference at $P < 0.05$. (b) Three linear regression models (early: day 0-19, mid: 22-42, late: 49-63) of *ln* transformed biomass of *J. vulgaris* in both sterilized and live soil. The two wztrapolated dashed parts of the lines are based on the linear regression models for day 0-19 and 49-63 (c) Mean slope (± SE) of the linear

regression lines in (b). Differences between the slopes for live soil and sterilized soil were tested for significance with a t-test. * indicates $P < 0.05$.

The effect of time of inoculation before planting on the growth of *J. vulgaris*

Experiment 3: Plants produced less biomass in inoculated soils than in sterilized soil (Fig. 4a). For young plants (0-21 days) the relative growth rate in sterilized soil was significantly larger than that for live-0, live-1, live-2 or live-4 soil. Relative growth rates did not differ for mid-aged plants between live-0, live-1 and live-2 soil. Interestingly, relative growth rates of plants from live-4 soil for the mid-aged period were significantly higher than the relative growth rate of plants in sterilized soil (Fig. 3c; Table S3). Timing of the inoculation did affect the relative growth rates of plants in the early phase (0-21 days). The longer the time between inoculation and planting the lower the relative growth rate of young plants was ($R^2 = 0.99$, $P < 0.05$, $df = 3$). This was no longer true for the mid-aged period ($R^2 = 0.71$, $P = 0.15$, $df = 3$). These results were largely backed up by the backward sequential regression, which showed that relative growth rates were only higher for plants grown in the sterilized soil if very young plant ages were included. Especially for the live-4 soil the relative growth rate was even higher for plants grown in live soil when only older plants were included (Table S3).

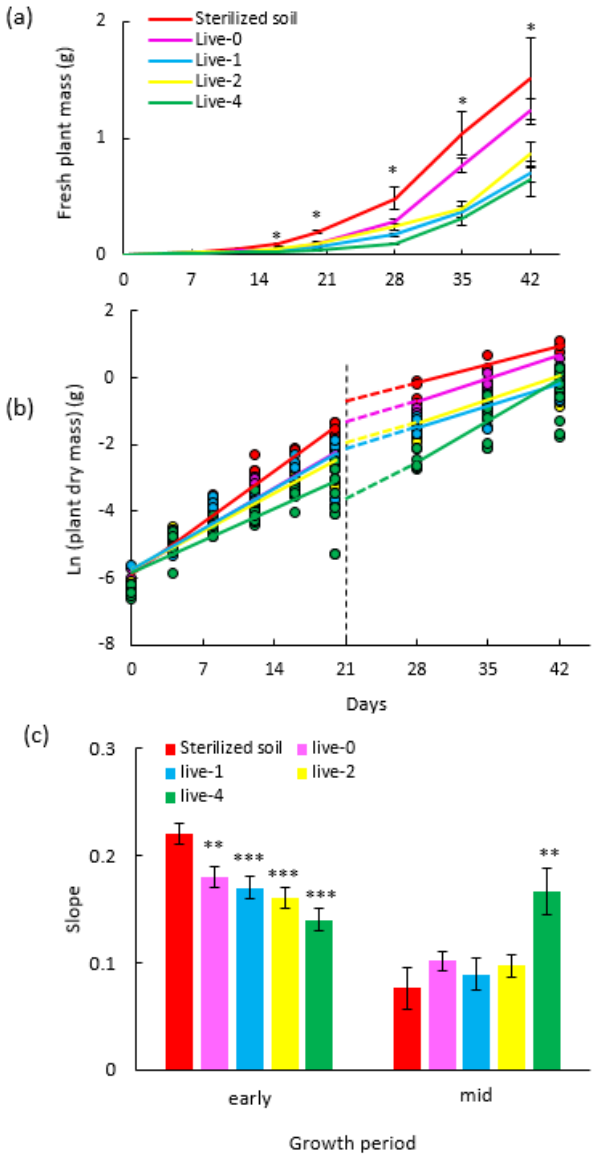

Fig. 4 Experiment 3. Plant growth of *J. vulgaris* in sterilized soil and in live soil 0, 1, 2 or 4 weeks before planting (live-0, live-1, live-2 and live-4). (a) Mean (± SE) fresh biomass of *J. vulgaris* in sterilized and live soil over 42 days. For each time point differences between the biomass of the plants in the sterilized soil and overall live soil (combining four live soils as an overall live soil treatment) were tested for significance with a t-test, * indicates a significant difference at $P < 0.05$. (b) Two linear regression

models (early: 0-20, mid: 28-42) of *ln* transformed fresh biomass of *J. vulgaris* in sterilized soil and four live soils. The extrapolated dashed parts of the lines are based on the linear regression models of day 28-42. (c) Mean slope (± SE) of linear lines in (b). Differences between the slopes for live-soil and sterilized soil were tested for significance with a t-test. *** indicates significant difference at $P < 0.001$; ** indicates significant difference at $P < 0.01$.

Discussion

In this study, we report the results of three experiments in which we measured the growth of *J. vulgaris* to test how the effects of soil microbial communities on plant growth change over time. We found a consistent negative effect of the soil microbial community in all three experiments. Biomass was larger in sterilized soil than in live soil. However, analyses of the *ln* transformed data, show that the relative growth rates were significantly higher in sterilized soil than in live soil only for young plants, and not for mid-aged plants. Moreover, in experiment 2, which was continued for a longer period, older plants even had a higher relative growth rate in the live soil. Hence, all data sets showed that the negative effects of soil inoculation on plant mass appear to extend during a long period, but arise from the negative effects that occur in the first weeks after planting when plants have only obtained less than 5% of the mass they obtain after 42 days.

We observed a consistent negative effect of live soil containing a natural soil microbial community in all three experiments. It is plausible that this was due to a net pathogenic effect of the soil microbial community on plant growth (Klironomos, 2002; Joosten et al., 2009; Harrison and Bardgett, 2010; Cortois et al., 2015). This hypothesis has been widely verified in other studies. For example, bacterial microbes such as *Ralstonia solanacearum, Agrobacterium tumefaciens*, *Erwinia amylovora and Streptomyces scabies* have been frequently isolated from natural soils (Curl et al., 1998; Michel et al., 1998; Gómez et al., 2017; Sharifazizi et al., 2017). These pathogenic microbes can adversely affect plant health and production (Huang et al., 2013; Cesarano et al., 2017). Several studies have indicated that soil microbes

compete with plants for available nutrients in the soil, and this could also result in negative effects on plant growth in inoculated soil (Bardgett et al., 2003; Fontaine et al., 2003; Dunn et al., 2006). However, in our study, we grew plants in a nutrient-rich environment by supplying a nutrient solution, and hence we argue that it is unlikely that the negative effect of live soil on plant growth was due to plant-microbe competition for nutrients. In Chapter 2, application of SA mitigated the negative effects of the live soil on the growth of *J. vulgaris*, in combination with the fact that activation of SA-dependent signaling pathways leads to the expression of pathogenesis-related proteins (PRP) contributing to resistance (Glazebrook, 2005; Spoel et al., 2007), this together suggests that the negative soil effect on plant growth was due to microbial pathogens.

Our study exemplifies that the negative effects of soil inoculation on plant mass can extend over the entire growth period, even though the differences are due to negative effects that occur during the first weeks after planting. There are several explanations for the observation that older plants do not exhibit a negative response to live soils. First, younger plants or seedlings may be more vulnerable and susceptible to pathogenic microbes in the soil than older plants with well-developed root systems (Packer and Clay, 2000). Root development plays an important role for plants in suppressing soil-borne pathogens (Watt et al., 2006; Emmett et al., 2014), and is correlated with soil abiotic or biotic characteristics (Kardol et al., 2013; Arrigoni et al., 2018; Bezemer et al., 2018). Herms and Mattson (1992) demonstrated that plants have to invest in their roots first before they can defend themselves against biotic stress. Hence, it may take a while for plants to build-up their defense systems (Raaijmakers et al., 2009; Hayat et al., 2010). Alternatively, it is well established that plants influence the soil microbial community during growth and hence, it is also possible that the differences in the response of younger and older plants to live soil is due to changes that have occurred in the soil microbial community. Previous work with the same plant species, *J. vulgaris*, where seedlings were planted in soil in which plants of the same species had been grown first, showed that the differences between responses of young and old plants are likely related to the sensitivity of plant stages and not due to changes in the soil community. Young *J. vulgaris* exhibited a strong

negative conspecific feedback, but this effect diminished over time and became neutral in older plants (Bezemer et al., 2018).

Interestingly we observed that the longer ago the soil was inoculated the stronger the negative effect of the inoculum on plant growth. This also indicates that the negative effects of live soil on plant growth that are commonly observed for this plant species are mediated by the soil microbial community and the variation that is typically observed in plant growth experiments may result from the different densities of soil-borne microbes. We expect that the oldest inoculated live soil contained the highest density of pathogenic microbes, leading to a stronger negative effect on plant growth (Pernilla et al., 2010; Dudenhöffer, et al., 2018). However, in this study, we did neither quantify the microbial density in the soil nor measure plant defense-related compounds such as salicylic acid, or pyrrolizidine alkaloids, and we suggest future work should focus on these two aspects.

In conclusion, our results indicate that live soil negatively affected plant growth. In most cases the difference between plant biomass of plants grown in sterilized soil and live soil increased during the entire experiment. However, the relative growth rates of plants in the sterilized soil and live soil only differed for young plants. Moreover, there was a negative correlation between the time of soil inoculation before planting and the relative growth rate of *J. vulgaris* plants, but for all incubation periods the negative effects were only present for young plants. Hence, our results suggest that young plants (≤ 21 days) or seedlings are most sensitive to soil pathogens while older plants (≥ 22 days) are no longer affected. Our study highlights the importance of examining relative growth rates rather than final biomass to estimate the effects of soil microbial communities on plants.

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Supplementary data

Table S1. Sequential regression analysis of *ln* transformed dry plant mass of *J. vulgaris* in experiment 1 in sterilized and live soil. The slopes were calculated backward sequentially. Slope1 and slope2 represent slopes in sterilized soil and live soil, respectively. SE indicates standard error, *df* means degrees of freedom. A t-test was used to determine significance between the two slopes. *** represents $P < 0.001$.

	Day range	slope1	SE1	slope2	SE2	*df*	*t*-value	*P*
Slopes for sequential backward regression	42-49	0.11	0.02	0.16	0.03	36	1.60	ns
	35-49	0.12	0.01	0.13	0.01	53	1.31	ns
	28-49	0.16	0.01	0.13	0.01	73	-2.86	ns
	21-49	0.14	0.01	0.12	0.01	93	-1.55	ns
	14-49	0.15	0.01	0.12	0.01	113	-4.54	***
	7-49	0.16	0.00	0.12	0.00	131	-6.85	***

Table S2. Sequential regression analysis of *ln* transformed dry plant mass of *J. vulgaris* in experiment 2 in sterilized and live soil. The slopes were calculated backward sequentially. Slope1 and slope2 represent slopes in sterilized soil and live soil. SE indicates standard error, *df* means degrees of freedom. A t-test was used to determine significance between the two slopes. * represents $P < 0.05$.

	Day range	slope1	SE1	slope2	SE2	*df*	*t*-value	*P*
Slopes for sequential backward regression	56-63	0.02	0.02	0.11	0.02	28	-2.61	*
	49-63	0.04	0.01	0.09	0.01	44	0.01	*
	42-63	0.08	0.01	0.11	0.01	60	-2.32	*
	35-63	0.09	0.01	0.11	0.01	76	-1.99	*
	28-63	0.10	0.00	0.12	0.00	92	-2.05	*
	22-63	0.11	0.00	0.12	0.00	108	-1.20	ns
	19-63	0.12	0.00	0.13	0.00	124	-1.62	ns
	16-63	0.13	0.00	0.13	0.00	140	-1.34	ns
	13-63	0.13	0.00	0.14	0.00	155	1.59	ns
	10-63	0.14	0.00	0.14	0.00	171	1.02	ns
	7-63	0.14	0.00	0.15	0.00	187	0.52	ns
	4-63	0.15	0.00	0.15	0.00	205	-0.19	ns
	0-63	0.17	0.00	0.17	0.00	223	-0.59	ns

Table S3. Sequential regression analysis of *ln* transformed fresh plant mass of *J. vulgaris* in experiment 3 in sterilized and inoculated soil 0, 1, 2 or 4 weeks before planting (live-0, live-1, live-2 and live-4). The slopes were calculated backward sequentially. Slope1 and slope2 represent slopes in sterilized soil and live soil respectively. SE indicates standard error, *df* degrees of freedom. A t-test was used to determine significance between the two slopes for each combination of sterilized and live soil. * represents $P < 0.05$, ** represents $P < 0.01$, *** represents $P < 0.001$. Note that the slopes for the sterilized soil are used for comparison with the slopes of the live-0, live-1, live-2 and live-4 and are thus represented in the table 4 times.

Backward sequential slope calculation

	Day range	slope1	SE1	slope2	SE2	*df*	*t*-value	*P*
	35-42	0.04	0.04	0.07	0.02	28	0.48	ns
	28-42	0.08	0.02	0.10	0.01	44	1.09	ns
	20-42	0.09	0.01	0.12	0.01	60	2.77	*
	16-42	0.10	0.01	0.13	0.01	76	2.64	*
	12-42	0.11	0.01	0.12	0.01	92	1.57	ns
	8-42	0.12	0.01	0.13	0.00	108	1.14	ns
	4-42	0.13	0.01	0.13	0.00	124	0.53	ns
Live-0	0-42	0.15	0.01	0.15	0.00	140	-0.31	ns
	35-42	0.04	0.04	0.12	0.04	28	1.35	ns
	28-42	0.08	0.02	0.09	0.02	44	0.49	ns
	20-42	0.09	0.01	0.10	0.01	60	0.86	ns
	16-42	0.10	0.01	0.10	0.01	76	0.05	ns
	12-42	0.11	0.01	0.11	0.01	92	-0.04	ns
	8-42	0.12	0.01	0.11	0.00	108	-1.22	ns
	4-42	0.13	0.01	0.12	0.00	124	-2.04	*
Live-1	0-42	0.15	0.01	0.13	0.00	140	-2.32	*
	35-42	0.04	0.04	0.10	0.03	28	1.02	ns
	28-42	0.08	0.02	0.10	0.01	44	0.86	ns
	20-42	0.09	0.01	0.11	0.01	60	1.51	ns
	16-42	0.10	0.01	0.10	0.01	76	0.69	ns
	12-42	0.11	0.01	0.11	0.00	92	-0.21	ns
	8-42	0.12	0.01	0.11	0.00	108	-1.75	ns
	4-42	0.13	0.01	0.11	0.00	124	-2.99	**
Live-2	0-42	0.15	0.01	0.13	0.00	140	-3.04	**
	35-42	0.04	0.04	0.16	0.05	26	1.67	ns
	28-42	0.08	0.02	0.17	0.02	40	2.94	**
	20-42	0.09	0.01	0.15	0.02	55	3.59	***
	16-42	0.10	0.01	0.13	0.01	71	2.34	*
	12-42	0.11	0.01	0.13	0.01	87	1.47	ns
	8-42	0.12	0.01	0.11	0.01	103	-0.97	ns
	4-42	0.13	0.01	0.12	0.01	119	-1.14	ns
Live-4	0-42	0.15	0.01	0.13	0.01	135	-2.19	*