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The negative effects of soil microorganisms on plant growth only extend to the first weeks

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Abstract

Soil biotic communities can strongly impact plant performance. In this paper, we ask the question: how long-lasting the effect of the soil microbial community on plant growth is. We examined the plant growth rates at three stages: early, mid and late growth. We performed two growth experiments with *Jacobaea vulgaris*, which lasted 49 and 63 days in sterilized soil or live soil. 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* with four different timing treatments. In all experiments, differences in biomass of plants grown in sterilized soil and live soil increased throughout the experiment. Also, the relative growth rate of plants in the sterilized soil was only significantly higher than that of plants in the live soil in the first two to three weeks. In the third experiment, plant biomass decreased with increasing time between inoculation and planting. Overall, our results showed that plants of *J. vulgaris* grew less well in live soil than in sterilized soil. The negative effects of soil inoculation on plant mass appeared to extend over the whole growth period but arise from the negative effects on relative growth rates that occurred in the first weeks.

Keywords plant–soil interactions, relative growth rate, plant performance, pathogenic soil microbial community

土壤微生物对植物生长的负面影响只延续到最初几周

摘要：土壤微生物群落可以显著影响植物的生长表现。在本文中，我们提出一个问题：土壤微生物群落对植物生长的影响可以持续多久。我们监测了早期、中期和晚期3个阶段的植物生长速率，在无菌土壤或活土壤中对一种菊科植物疆千里光(*Jacobaea vulgaris*)进行了两次分别为49天和63天的生长实验。在第3个实验中，我们用4种不同的时间处理方法研究了种植前土壤接种时间对该植物相对生长速率的影响。研究结果表明，3个实验中，在无菌土壤和活土壤中生长的植物的生物量差异都增加了。此外，在前2–3周，灭菌土壤中植物的相对生长速率仅显著高于活土壤中植物的相对生长速率。在第3个实验中，植物生物量随着接种和种植之间时间的增加而减少。总体而言，这些结果表明，疆千里光在无菌土壤中的生长优于在活土壤中。土壤接种对植物生物量的负面影响似乎可以延伸到整个生长期，但源于最初几周发生的对相对生长速率的负面影响。

关键词：植物–土壤相互作用，相对生长速率，植物生长表现，病原土壤微生物群落

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; Erktan *et al.* 2018; Teste *et al.* 2017). The soil microbiome is an important driver of plant performance. Soil microbial species e.g. pathogenic organisms, plant-growth-promoting rhizobacteria (like *Pseudomonas* and *Burkholderia*) and arbuscular mycorrhizal fungi, play an active role in modifying the development of plants (Arora and Mishra 2016; Artursson *et al.* 2006; Gil-Martínez *et al.* 2018; Johnson *et al.* 1997). Evidence has mounted that the effects of the soil microbial community on plant growth in laboratory experiments is mostly negative for many species (Cortois *et al.* 2016; Mangan *et al.* 2010; van de Voorde *et al.* 2012).

One potential explanation for the negative effect of soil microbes on plant performance is that microbes and plants compete for nutrients (Kardol *et al.* 2013). Alternatively, pathogens may accumulate in the soil over time, eventually resulting in a negative overall effect on plant performance (Dobson and Crawley 1994; Jacoby *et al.* 2017; Mordecai 2011; Raaijmakers *et al.* 2009; van der Putten *et al.* 2013; Wardle *et al.* 2004). 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 6–8 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 artifact of the experimental design (Jing *et al.* 2015; Smith and Reynolds 2012; van de Voorde *et al.* 2012). It is also possible, however, that the pathogenic effects of the soil microbial community only last for a short period because (i) only seedlings are susceptible or (ii) 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 focused on the effect of the soil microbial community on final plant biomass (Anacker *et al.* 2014; Bezemer *et al.* 2013; van de Voorde *et al.* 2012). 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 to pathogenic microbes in the soil (Packer and Clay 2000). In contrast, older plants with a more developed root system are typically less vulnerable (Bezemer *et al.* 2018; Kardol *et al.* 2013).

The increase in plant biomass is not only determined by growing conditions but also by the biomass of the plant itself. Effects on plant growth that occur during early life stages can, therefore, 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 (solid line). Plants in live soil either grow with a constant relative growth rate lower than that of the plants in the live soil (gray dashed 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 (black dashed line). In the latter case, although the effect of the soil microbial community only is present until t_1, differences in plant mass continue to increase (Fig. 1b). Hence, to study the effect of soil microbes on plants, it is important to also 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) plant relative growth rates are smaller in live soil than in sterilized soils, (ii) the negative effect on relative growth 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.

Previously, we observed in experiments with ample nutrient supply that the negative effect of the soil microbial community on plant growth was 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. An important question is therefore how long the negative effects of the soil microbial community on plant growth lasts. We used *Jacobaea vulgaris* to test these hypotheses. *Jacobaea vulgaris* is native to The Netherlands. In a former experiment, we found that

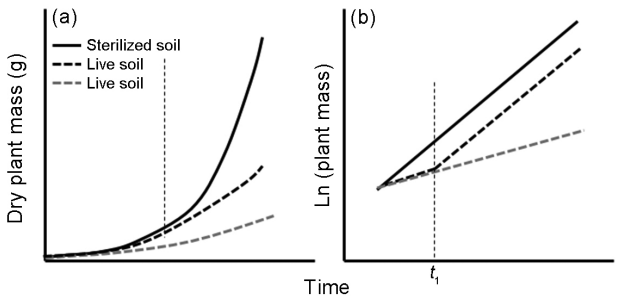

Figure 1: Conceptual figures showing plant mass of *J. vulgaris* in both sterilized soil and live soil over time. (**a**) The biomass of plants plotted against time. (**b**) Ln biomass plotted against time. The regression coefficients (slopes) in (b) are equal to the relative growth rates of the plants. The growth rate in sterilized soil (solid line) is higher than that in live soil (gray dashed line) (hypothesis 1) and this difference is maintained during the entire plant growth period. The black dashed line indicates an initial lower relative growth rate of plants in the live soil (a lower slope in Fig. 1b) but at t_1 these plants obtain an equal relative growth rate as plants in the live soil (a similar slope in Fig. 1b, hypothesis 2). Note that even when relative growth rates become equal after an initial difference in the early stage of life (the solid line and the black dashed line in Fig. 1a) the difference in absolute biomass continues to increase after that period (the solid line and the black dashed line in Fig. 1b). The gray dashed lines show a case in which both the absolute and relative growth are lower for plants in live soil.

plant mass of *J. vulgaris* growing in soil containing a live microbial community was 66% lower than when plants were grown in sterilized soil (unpublished data). This negative effect of live soil on plant growth is in line with previous findings (Kos *et al.* 2015; van de Voorde *et al.* 2012; 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 (Joosten *et al.* 2009; Steiner 1980). 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 of sterilized soil impacts the growth of *J. vulgaris*.

MATERIALS AND METHODS

Jacobaea vulgaris (common ragwort) was used as plant species. We chose this species because it is a common species in The Netherlands i.e. strongly affected by plant–soil interactions (Bezemer *et al.* 2013; van de Voorde *et al.* 2011, 2012). 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 (humidity 70%, light 16 h at 20 °C, dark 8 h at 20 °C) for the duration of germination.

Soil

Topsoil was collected at Meijendel 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-L 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’. Per pot contains only one plant. 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 16 h at 20 °C, dark 8 h 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 seven 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 L demineralized water, and a stock solution with microelements (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 L demineralized water). Ten mL of each stock solution was diluted in 1 L 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* over time was performed starting with 1-week-old seedlings, two soil treatments and eight harvesting time points over 7 weeks. The harvests were on days 0 (germinated 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 1 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 days 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 1 week, and dry mass was determined.

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

Experiment 3

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 16 h at 20 °C, dark 8 h at 20 °C) to enable different buildup 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 6 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 five treatments × nine harvests × eight 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. The regression coefficient for this relationship provides an estimate for the relative growth rates of the plants. Plant growth was divided into three stages: early growth (0–21 days), mid growth (22–42 days) and late growth (43–63 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 analysis 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 $\left(t = \frac{\text{Slope1} - \text{Slope2}}{\text{SQRT}(\text{SE1}^2 + \text{SE2}^2)}\right)$ in IBM SPSS Statistics 25.

Relative growth rates were calculated as: $\text{rgr} = (\ln \text{biomass2} - \ln \text{biomass1})/(time2 - time1)$ and these results are presented in Supplementary Fig. S1. The effects of the soil treatments (sterilized and live soil) and harvest time point on the plant biomass of *J. vulgaris* were tested using a two-way ANOVA with ln-transformed plant dry mass as a dependent variable and soil (2 levels) as a fixed factor and harvest time point (7 levels for Experiment 1, 14 levels for Experiment 2, 9 levels for Experiment 3 in each live soil) as a continuous factor. Differences between treatments were compared with a Tukey *post hoc* test.

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 the live soil (Fig. 2). For young plants (0–21 days) the relative growth rate (slope in Fig. 2b and 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 and 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 49 and 22 days (Supplementary Table S1). The difference of plant dry biomass in response to live soils among the different harvest time points was reflected by a highly significant interaction between soil × harvest time in the two-way ANOVA (Supplementary Table S4, Experiment 1). The relative growth rates of Experiment 1 differed among different harvest time points (Supplementary Fig. S1-1).

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 and c). Backward regression showed that the relative growth rate was higher for the plants in the live soil for the period 63–28 days. If younger ages were included differences were no longer significant (Supplementary Table S2).

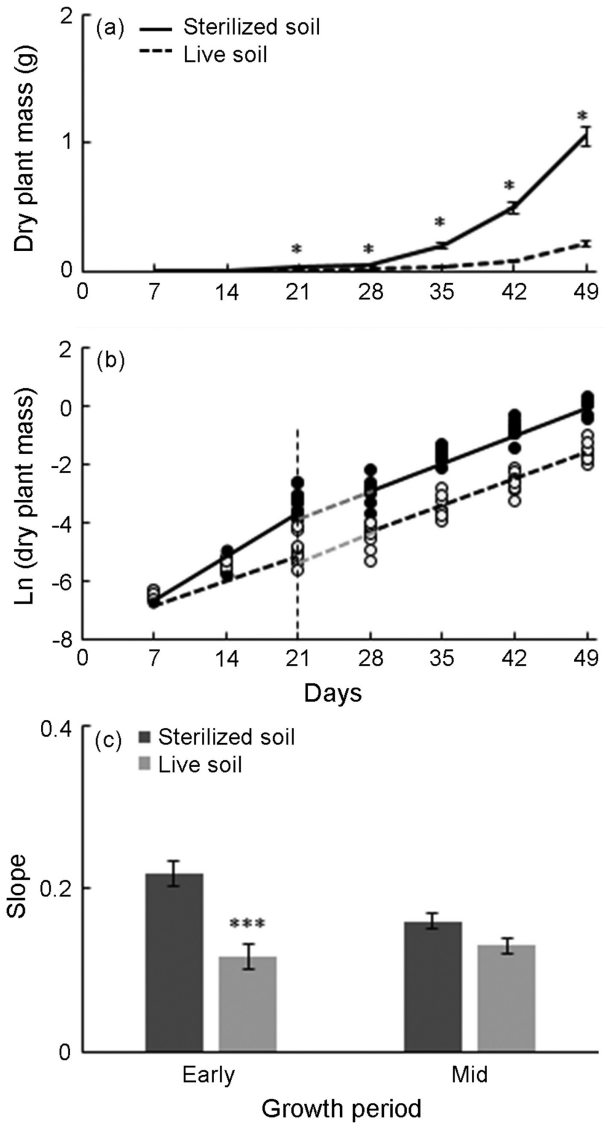

Figure 2: Experiment 1. (**a**) Mean (±SE) biomass of *J. vulgaris* in sterilized and live soil over 49 days. For each time point, differences between the biomass of the plants in the two soils were tested for significance with a *t*-test, * indicates a significant difference ($P < 0.05$). (**b**) Two linear regression models (days 7–21 and 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 days 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 *t*-test. *** indicates $P < 0.001$.

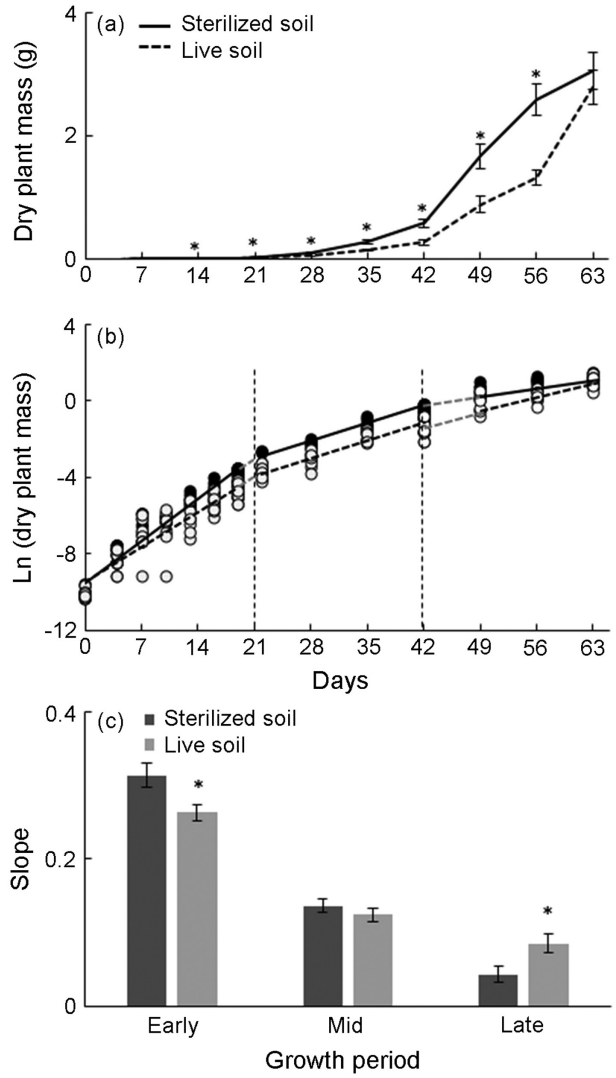

Figure 3: Experiment 2. (**a**) Mean (±SE) of dry plant mass of *J. vulgaris* in sterilized and live soil over 63 days. For each time point, differences between the biomass of the plants in the two soils were tested for significance with a *t*-test, * indicates a significant difference at $P < 0.05$. (**b**) Three linear regression models (days 0–19, 22–42 and 49–63) of ln-transformed biomass of *J. vulgaris* in both sterilized and live soil. The two extrapolated dashed parts of the lines are based on the linear regression models for days 0–19 and 49–63. (**c**) 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 difference of plant dry biomass in response to live soils among the different harvest time points is not reflected by a significant interaction between soil × harvest time in the two-way ANOVA (Supplementary Table S4, Experiment 2). The relative growth rates of Experiment 2 differed among different harvest time points (Supplementary Fig. S1-2).

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 rate of plants from live-4 soil for the mid-aged period was significantly higher than the relative growth rate of plants in sterilized soil (Fig. 3c; Supplementary Table S3). Timing of the inoculation did affect the relative growth rate 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 lives soil when only older plants were included (Supplementary Table S3). The difference of plant dry biomass in response to live-0, live-1, live-2 and live-4 soils among the different harvest time points was reflected by a highly significant interaction between soil × harvest time in the two-way ANOVA (Supplementary Table S4, Experiment 3-a, 3-b, 3-c and 3-d). The relative growth rates of Experiment 3 were largely different among sterilized soil and live soils, but the overall trends of the relative growth rates among live-0, live-1, live-2 and live-4 were similar (Supplementary Fig. S1-1).

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

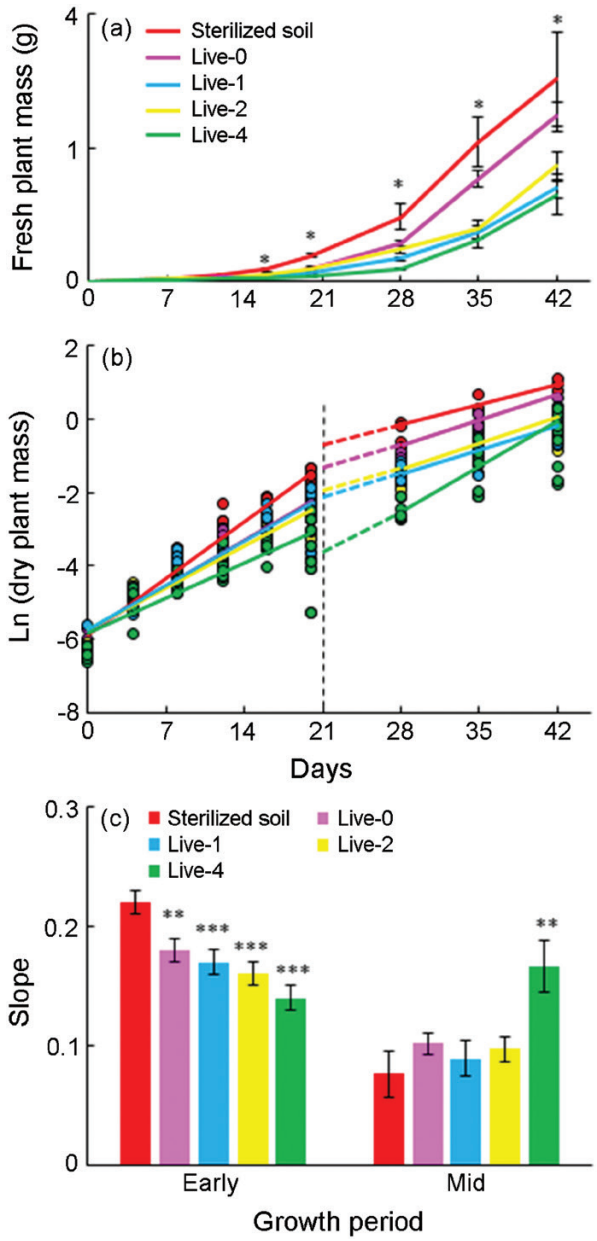

Figure 4: Experiment 3. Plant growth of *J. vulgaris* in sterilized soil and in live soil 0, 1, 2 or 4 weeks before planting (live-0, live-1, live-2 and live-4). (**a**) Mean (±SE) fresh biomass of *J. vulgaris* in sterilized and live soil over 42 days. For each time point differences between the biomass 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 (0–20 and 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 days 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 $P < 0.01$.

continued for a longer period, older plants even had a higher relative growth rate in the live soil. Hence, all datasets showed that the negative effects of soil inoculation on plant mass appear to extend over 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.

It is plausible that the observed effect was due to a net pathogenic effect of the soil microbial community on plant growth (Cortois *et al.* 2016; Harrison and Bardgett 2010; Joosten *et al.* 2009; Klironomos 2002). This hypothesis has been widely verified in other studies. For example, bacterial species such as *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Erwinia amylovora* and *Streptomyces scabies* have been frequently isolated from natural soils (Curl *et al.* 1988; Gómez Expósito *et al.* 2017; Michel and Mew 1998; Sharifazizi *et al.* 2017). These pathogenic microbes can adversely affect plant health and production (Cesarano *et al.* 2017; Huang *et al.* 2013). 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; Dunn *et al.* 2006; Fontaine *et al.* 2003). 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 an unpublished study, 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 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 (≥22 days) do not exhibit a negative response to live soils. First, younger plants (≤21 days) 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 (Emmett *et al.* 2014; Watt *et al.*

2006) and is correlated with soil abiotic or biotic characteristics (Arrigoni *et al.* 2018; Bezemer *et al.* 2018; Kardol *et al.* 2013). 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 buildup their defense systems (Hayat *et al.* 2010; Raaijmakers *et al.* 2009). 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).

The results of Experiment 1 suggest that the negative response of plant growth to live soil are due to a time lag in plant biomass accumulation during the early stage (≤21 days) of plant development. Interestingly, data in Experiment 2 showed plants were able to 'catch-up' and that plants exhibited compensatory increased growth rate to obtain similar final biomass under both treatments. Altogether, our results seem to suggest that there was a delayed start (a prolonged 'lag phase') to the log (exponential) phase of the associating with a net-negative soil community. To confirm this, further studies should examine changes in relative growth rates of single plants (i.e. growth measured repeatedly on the same individuals) and also do this for an extended growth period.

Interestingly, we observed that the longer the time since 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. Variation i.e. typically observed in plant growth experiments with this species 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 (Dudenhöffer, *et al.* 2018; Pernilla *et al.* 2010). However, in this study, we did not 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 growth of *J. vulgaris*. 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 *J. vulgaris* plants (≤21 days) or seedlings are most sensitive to soil pathogens while older plants (≥22 days) are no longer affected.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: Sequential regression analysis of ln-transformed dry plant mass of *J. vulgaris* in Experiment 1 in sterilized and live soil.

Table S2: Sequential regression analysis of ln-transformed dry plant mass of *J. vulgaris* in Experiment 2 in sterilized and live soil.

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).

Table S4: Two-way ANOVA of ln-transformed plant dry mass of *J. vulgaris* in Experiments 1, 2 and 3 in sterilized and live soil over 49, 63 and 42 days.

Figure S1: Results of the growth rate (rgr) of *J. vulgaris* in sterilized and live soil for Experiments 1–3.

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