Linalool-triggered plant-soil feedback drives defense adaptation in dense maize plantings

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RESEARCH ARTICLE SUMMARY

PLANT COMMUNICATION

Linalool-triggered plant-soil feedback drives defense adaptation in dense maize plantings

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INTRODUCTION: Planting crops more densely increases overall yields, but it also raises the risk of pest and pathogen outbreaks. Although plants can modify their architecture to adapt to crowded conditions, how they adjust their immune responses remains largely unknown. Understanding how plants manage these trade-offs is critical for sustainable agriculture, especially in the context of increasing global food demands.

RATIONALE: Plants release chemical cues, such as volatiles, that inform neighbors of environmental conditions. One such compound, linalool, is a constitutively emitted leaf volatile in maize and other grasses. We hypothesized that linalool could act as a signal in densely planted fields, triggering plant-soil feedback that prepares neighboring plants for potential biotic stress. We explored how linalool shapes root signaling, soil microbiota, and ultimately plant defense and growth.

RESULTS: Field surveys revealed that maize plants in the inner rows of densely planted fields suffered less herbivore damage than those at the edges but that they also had reduced growth. Laboratory soil–transplantation experiments confirmed that soils conditioned by high-density plantings decreased plant biomass while enhancing resistance to insects, nematodes, and pathogens. These effects extended across genotypes and species.

Volatile profiling identified linalool as a key compound increasing with planting density. Exposure of maize to synthetic linalool reproduced the feedback effects, which required the presence of a living plant. Mechanistically, linalool activated jasmonate signaling in roots and up-regulated genes that drive the biosynthesis and exudation of the specialized metabolite HDMBOA-Glc. This exudate reshaped the rhizosphere microbiome, selectively enriching bacteria that suppressed plant growth but increased resistance in subsequently grown plants. Soil sterilization and microbial inoculation confirmed that these microbes were essential for the feedback loop.

In plants grown in linalool-conditioned soil, defense-related signaling, particularly salicylic acid signaling, was up-regulated, whereas growth-promoting metabolic pathways were down-regulated. Plants lacking salicylic acid signaling did not show growth-defense trade-offs, confirming salicylic acid's role in expressing the feedback-triggered defense.

CONCLUSION: This study uncovers a volatile-triggered feedback mechanism through which maize adapts its defense in crowded environments. The constitutive emission of linalool primes neighboring plants by activating root jasmonate signaling, promoting HDMBOA-Glc exudation, and altering the rhizosphere microbiome. This, in turn, leads to elevated defense and suppressed growth in subsequent plants through salicylic acid signaling. These findings shed light on how plants integrate aboveground cues and belowground processes to optimize defense in high-density settings. Harnessing this natural defense pathway through breeding, microbial inoculants, or synthetic biology could enable the development of crops that are more resilient and require fewer chemical inputs. □

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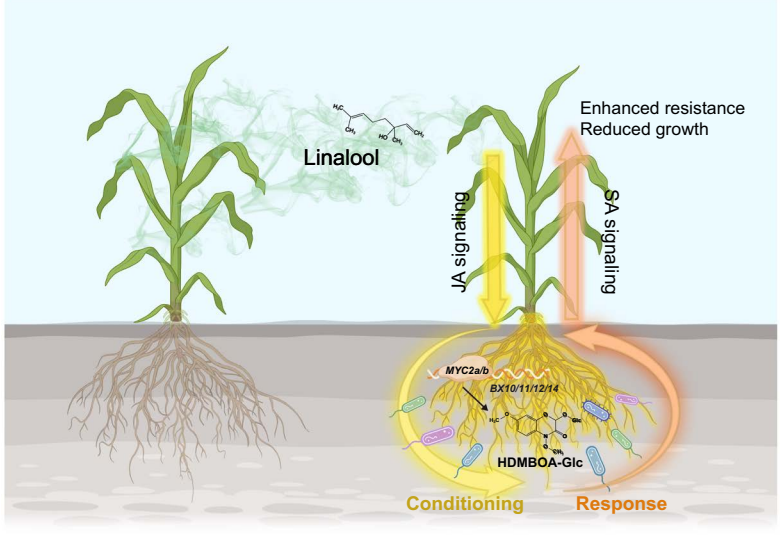

Model illustrating how dense planting triggers plant-soil feedback to enhance maize resistance. High-density planting increases canopy linalool, which activates systemic jasmonic acid (JA) signaling and HDMBOA-Glc exudation from roots. These exudates selectively enrich rhizosphere bacteria that induce salicylic acid (SA) signaling, thereby enhancing maize resistance. This defense-promoting feedback comes at a cost to maize growth. Some components of this figure were created by BioRender.com.

PLANT COMMUNICATION

Linalool-triggered plant-soil feedback drives defense adaptation in dense maize plantings

Dongsheng Guo[1,2]†, Zilin Liu[1,2]†, Jos M. Raaijmakers[3,4]†, Yachun Xu[1], Jinghui Yang[1], Matthias Erb[5], Jiabao Zhang[6], Yong-Guan Zhu[7], Jianming Xu[1]*, Lingfei Hu[1,2]*

High planting density boosts crop yields but also heightens pest and pathogen risks. How plants adapt their defenses under these conditions remains unclear. In this study, we reveal that maize enhances its defense in high-density conditions through a plant-soil feedback mechanism triggered by the leaf volatile linalool. Linalool activates jasmonate signaling in neighboring plants and promotes root exudation of benzoxazinoids, especially 2-(2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one)-β-d-glucopyranose (HDMBOA-Glc). These exudates in turn reshape the rhizosphere microbiome composition to favor growth of specific bacterial taxa that trigger broad-spectrum resistance, albeit at the cost of maize growth. This microbiome-driven feedback loop is governed by salicylic acid signaling. Our findings uncover intricate chemical signaling in high-density cropping, which is instrumental for improving soil health and designing sustainable strategies that balance the trade-off between plant growth and defense.

Enhancing crop yields is crucial to ensuring global food security and achieving United Nations Sustainable Development Goal 2: Zero Hunger by 2030 (*1*). Increasing planting density was and still is a key agronomic strategy for maximizing crop productivity (*2*). Over the past few decades, this strategy has substantially contributed to yield improvements across regions including the United States, China, Brazil, and France (*3*). Research on dense planting has primarily focused on how plants adapt their phenotypic plasticity to thrive under high-density conditions. In maize (*Zea mays*), for example, high-density planting induces changes in both shoot and root architecture, including steeper leaf angles and more compact root systems to minimize competition for light and nutrients (*4*–*6*). These traits have been harnessed in breeding programs to develop high-density–tolerant cultivars aimed at further increasing yield potential (*7*, *8*).

Although dense planting enhances overall yield, it also heightens the risk of disease outbreaks and herbivore infestations, a factor that is often overlooked. Denser plantings can modify the microclimate, reducing air circulation and increasing moisture retention on plant surfaces, which fosters conditions favorable to pathogen proliferation (*9*, *10*). Moreover, closely spaced plants allow for the rapid spread of herbivores and pathogens (*11*, *12*). However, how plants adjust their defense mechanisms to mitigate increased vulnerability to pathogens and herbivores in dense planting conditions remains poorly understood. This knowledge gap limits the potential for fully optimizing dense planting practices for sustainable agriculture.

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Plant-plant interactions are crucial for shaping community composition and dynamics, particularly in high-density environments (*13*, *14*). Various environmental cues, such as light quality and quantity (*15*), root exudates (*16*), and leaf volatiles (*17*–*19*), provide essential information about the proximity and physiological status of neighbors, as well as potential threats. Clearly, the presence, intensity, and reliability of these cues are highly dependent on proximity: At high planting densities, neighbors are closer, making chemical signals stronger, whereas at low densities these cues may be too weak to trigger effective responses. In this context, could plants in high-density environments use these cues to mitigate the risks of disease and herbivory, ultimately benefiting the performance and fitness of the entire plant community?

We addressed this question by investigating the defense plasticity of maize plants in a dense planting environment. Our findings uncover an intricate mechanism by which plants adjust their growth and defenses through leaf volatile–mediated plant-plant interactions and plant-soil feedback, enabling adaptation to high-density planting conditions.

Results

Planting density affects plant growth and defense in the field through plant-plant interactions

To investigate whether planting density affects plant defense through plant-plant interactions, we conducted a herbivory survey across four independent maize fields. On the basis of the optimal maize planting density in China (*3*), Fields I and II were planted at a relatively low density (~60,000 plants ha^{-1}), whereas Fields III and IV were planted at a high density (~120,000 plants ha^{-1}). Each field was divided into inner sections and outer edges to assess spatial variation in plant growth and herbivory (Fig. 1A). Under high-density conditions, plant-plant interactions such as shading and reduced air circulation are typically more intense in the inner sections of crop stands than at the outer edges. In high-density fields (Fields III and IV), herbivore damage was significantly lower in the inner sections compared with the outer edges (Fig. 1B). Conversely, plant height, shoot biomass, grain yield per ear, and 1000-grain weight were greater at the edges than in the interior of these high-density fields (fig. S1). However, these patterns were absent in the low-density fields (Fields I and II) (Fig. 1B and fig. S1). These results suggest that plant-plant interactions play a critical role in modulating growth and defense in dense planting environments.

Planting density affects plant growth and defense through plant-soil feedback

To assess whether planting density influences plant growth and defense through plant-soil feedback, while excluding the direct effects of root-root interactions, we first cultivated individual maize plants in separate pots filled with field bulk soil until four-leaf stage in the greenhouse. These plants were then arranged at varying planting densities—50, 100, 150, and 200 plants m^{-2} (Fig. 1C). With increasing density, both photosynthetically active radiation and the red-to-far-red light ratio beneath the canopy declined (fig. S2), confirming that a high-density light environment was successfully established (*20*). After 3 days of density treatment, we removed the plants but retained the conditioned soils. New maize plants were then planted into these conditioned soils and spaced to prevent direct density effects on their growth and defense (Fig. 1C). This experimental design ensured that any observed differences in the growth or defense of new plants were attributed to plant-soil feedback rather than direct root-root interactions or immediate density effects.

We observed that as planting density during the conditioning phase increased, the shoot biomass of new maize plants during the response phase progressively decreased, with the highest biomass found in soils previously conditioned at 50 plants m^{-2} and the lowest at 200 plants m^{-2} (Fig. 1, D and E). Plants grown in soils conditioned at 200 plants m^{-2} also exhibited smaller root systems, shorter plant height, and lower

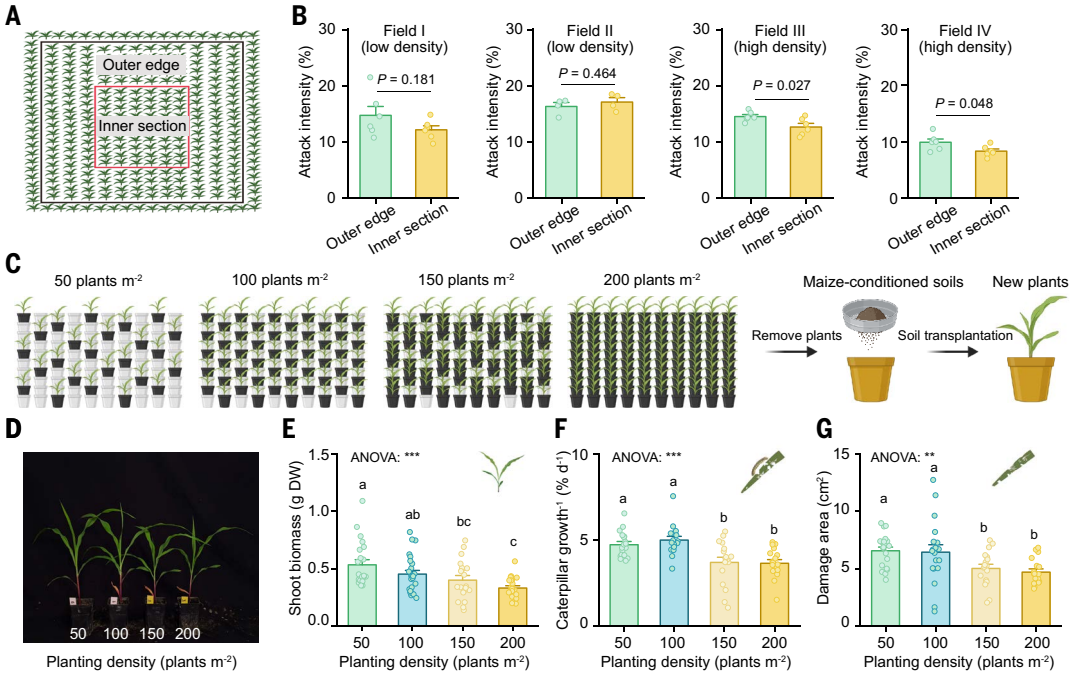

Fig. 1. Planting density enhances defense and reduces growth through plant-soil feedback. (**A**) Diagram of the herbivory survey conducted in maize fields. The field was divided into inner sections and outer edges as shown. (**B**) Herbivory attack intensities in the inner sections and outer edges of four independent maize fields [+ standard error (SE), $n = 4$ or 6 biological replicates; one biological replicate includes 100 to 256 individual plants]. Field I (660 m^2) and Field II (600 m^2) were planted with the maize cultivar Taiyanghua6 at a low planting density (~60,000 plants ha^{-1}). Field III (2100 m^2) and Field IV (2200 m^2) were planted at a higher density (~120,000 plants ha^{-1}). Data points represent individual replicate samples. The exact *P* values for each comparison are indicated in the figure (two-sided Student's *t* test). (**C**) Experimental design for soil transplantation trials. Individual maize plants were grown in separate pots until four-leaf stage. These plants were then arranged at varying planting densities for 3 days. White pots indicate bulk soil without a plant. After 3 days of density treatment, we removed the plants but retained the conditioned soils. The soils from each density treatment were homogenized and sieved. New maize plants were then planted into these conditioned soils and spaced to prevent direct density effects on their growth and defense. This figure was generated by the BioRender software. (**D**) Growth phenotype of new maize plants grown in soils previously conditioned by plants at varying densities. (**E** to **G**) Shoot biomass in grams, dry weight (g DW) (E), caterpillar weight gain in percent per day (% d^{-1}) (F), and leaf damage (G) of new maize plants grown in soils previously conditioned by plants at varying densities (+ SE, $n = 16$ to 24). Data points represent individual replicate samples. Different letters (a, b, and c) denote significant differences between treatments [analysis of variance (ANOVA) followed by multiple comparisons of false discovery rate (FDR)–corrected least squares means (LS means); $P < 0.05$]. Asterisks indicate overall ANOVA significance (***$P < 0.001$, **$P < 0.01$). Raw data, exact biological replicates, and *P* values for all comparisons in this figure are provided in Figshare (*62*).

chlorophyll content throughout their growth (fig. S3). By contrast, insect resistance of new maize plants showed an opposite trend, being lowest in soils conditioned at 50 plants m^{-2} and highest at 200 plants m^{-2} (Fig. 1, F and G, and fig. S4). Larvae of the leaf chewing herbivore fall armyworm (*Spodoptera frugiperda*) grew less and caused less damage to maize plants grown in soils conditioned at 150 and 200 plants m^{-2} (Fig. 1, F and G). Likewise, the root-knot nematode *Meloidogyne incognita* formed fewer galls on roots of plants grown in soils conditioned at 200 plants m^{-2} (fig. S4, A and B). Northern corn leaf blight disease caused by the fungus *Exserohilum turcicum* was also less prevalent in plants grown in soils previously conditioned at 200 plants m^{-2} (fig. S4, C and D). Similarly, replication and virulence of rice black-streaked dwarf virus, which causes maize rough dwarf disease, were significantly reduced in plants grown in soils conditioned at 200 plants m^{-2} (fig. S4, E and F). Taken together, these findings indicate that planting density triggers plant-soil feedback, resulting in decreased plant growth but enhanced defenses against above- and below-ground herbivores as well as fungal and viral pathogens.

Next, we asked whether the observed planting density–induced plant-soil feedback effects depend on the maize genotype. We planted three additional maize cultivars (B104, W22, and KN5585) in bulk soil at planting densities of 50 and 200 plants m^{-2} and subsequently grew each in its respective conditioned soil. High planting densities consistently decreased growth and enhanced resistance across all tested maize cultivars (fig. S5). To test whether the observed plant-soil feedback occurs across plant species, we cultivated maize plants alongside ryegrass (*Lolium perenne*) or barley (*Hordeum vulgare*) and assessed the feedback effects in maize-conditioned soils (fig. S6A). High-density planting with ryegrass or barley also decreased the growth and enhanced herbivore resistance of the subsequently grown maize plants (fig. S6, B to D). To test whether the observed feedback effects are specific to maize, we grew ryegrass or barley in soils previously conditioned by maize at densities of 50 or 200 plants m^{-2} (fig. S7A). Both ryegrass and barley exhibited enhanced herbivore resistance and reduced growth when grown in high-density, maize-conditioned soils (fig. S7, B and C). Lastly, we conditioned soils using ryegrass or barley themselves at low and high planting densities and found that these species also displayed increased resistance under high-density soil conditions (fig. S8). Together, these results demonstrate that planting density alters plant-soil feedback in a manner that suppresses plant growth while enhancing defense. These effects are consistent across maize genotypes and are functionally transferable between different plant species.

Leaf volatile linalool drives planting density–mediated plant-soil feedback

Dense planting may cause leaf volatile accumulation and canopy shade, both of which can influence the physiological status of neighboring plants (*15*, *21*). We hypothesized that these factors might drive plant-plant interactions and thereby affect new plants through plant-soil feedback. To this end, we profiled the leaf volatiles released by maize

plants grown at various densities in an open-air environment using solid-phase microextraction (fig. S9A). Linalool was the most pronounced plant volatile, and its concentration varied according to planting density (Fig. 2A and fig. S9, B and D). Linalool is constitutively released by maize leaves (*22*), and we further detected its emission in both ryegrass and barley (fig. S10), which suggests its broader occurrence across plant species. To evaluate the contribution of linalool to the observed plant-soil feedback effects on growth and defense, we constructed a dispenser releasing synthetic linalool. By adjusting the exposure distance, we simulated the physiological concentrations observed at different planting densities (fig. S9, C and D). We found that exposure to linalool at a distance of ~5 cm (Lin5) significantly enhanced herbivore resistance and reduced growth in newly planted maize (Fig. 2, B to D, and fig. S9, D and E). On the basis of these results, we adopted this exposure setup (referred to as the "Lin" treatment hereafter) for subsequent experiments.

Next, we used *tps2* mutant plants, which are deficient in linalool biosynthesis but still experience leaf shading under high-density conditions (Fig. 2E and fig. S11). New maize plants grown in the soils of high-density *tps2* mutants did not exhibit enhanced herbivore resistance or reduced growth (Fig. 2, F to H). However, when we supplemented the *tps2* mutants with physiological doses of linalool using dispensers, the feedback effects on growth and defense of new maize plants reappeared (Fig. 2, F to H). Taken together, these results indicate that the leaf volatile linalool, rather than leaf shading, is sufficient to trigger the plant-soil feedback mediated by dense planting.

Linalool triggers plant-soil feedback by inducing root JA signaling

We exposed soil to linalool in the absence of plants and subsequently assessed the soil's impact on the maize plants (fig. S12A). The results showed no changes in growth nor in herbivore resistance of the new plants (fig. S12, B to D), indicating that the presence of a receiver plant is essential for linalool-mediated plant-soil feedback. Considering that plant volatiles are known to influence phytohormone signaling (*23*), with putative effects on root physiology and thereby on plant-soil feedback, we measured phytohormone levels in maize roots after linalool exposure. We found that linalool exposure of receiver plants significantly increased jasmonic acid (JA) and JA-isoleucine (JA-Ile) levels in the roots, whereas levels of abscisic acid (ABA), salicylic acid (SA), and indole-3-acetic acid (IAA) remained unchanged (Fig. 3A). To further investigate the role of jasmonate signaling in plant-soil feedback, we used jasmonate-deficient *lox8* mutant plants as linalool-receiving plants. Compared with wild-type plants, *lox8* mutants displayed markedly reduced jasmonate levels in their roots, both with and without linalool exposure (fig. S13). Linalool exposure failed to induce plant-soil feedback effects on growth and herbivore resistance when *lox8* plants were used as conditioners (Fig. 3, B to D).

Because jasmonates can be exuded from roots into the rhizosphere (*24*), we also explored whether linalool triggers jasmonate exudation. However, quantification of jasmonate levels in the rhizosphere soil after linalool exposure revealed no significant changes (Fig. 3A). Taken together, these findings suggest that linalool-induced JA signaling in maize roots is critical for initiating plant-soil feedback but that it does not act through the direct exudation of jasmonates into the soil.

Linalool triggers plant-soil feedback through benzoxazinoid exudation

Benzoxazinoids (BXs) are exuded by plant roots into the soil, influencing plant growth and herbivore resistance (*25*). We hypothesized that linalool may regulate plant-soil feedback by regulating BX biosynthesis. To test this, we quantified BX levels in both the roots and rhizosphere

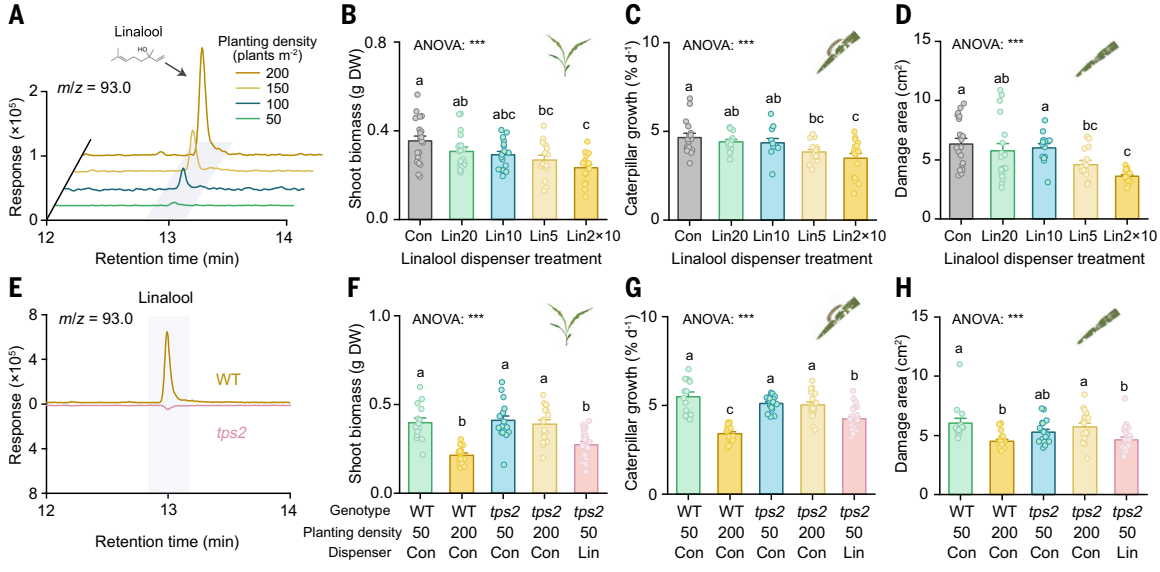

Fig. 2. Linalool drives planting density–mediated plant-soil feedback. (**A**) Selected gas chromotagraphy–mass spectrometry (GC/MS) ion chromatograms of volatiles from maize plants grown at various densities in an open-air environment using solid-phase microextraction (for detailed information of volatile profiles and linalool concentration, see fig. S9, B and D). *m/z*, mass/charge ratio. (**B** to **D**) Shoot biomass (B), caterpillar weight gain (C), and leaf damage (D) of new maize plants grown in soils previously conditioned by plants exposed to control (Con) or synthetic linalool (Lin) released by a dispenser (+ SE, $n = 12$ to 20) (for the schematic setup of maize plants exposed to dispensers, refer to fig. S9, C and E). Data points represent individual replicate samples. Different letters indicate significant differences between treatments (ANOVA followed by multiple comparisons of FDR-corrected LS means; $P < 0.05$). Asterisks indicate overall ANOVA significance (***$P < 0.001$). (**E**) GC/MS selected ion chromatograms of volatiles from wild-type (WT) and linalool-deficient mutant *tps2* (the detailed linalool amount in WT and *tps2* plants are presented in fig. S11). (**F** to **H**) Shoot biomass (F), caterpillar weight gain (G), and leaf damage (H) of new maize plants grown in soils previously conditioned by WT or *tps2* plants at planting densities of 50 or 200 plants m^{-2} or by *tps2* plants at 50 plants m^{-2} complemented with synthetic linalool via a dispenser (+ SE, $n = 14$ to 24). Data points represent individual replicate samples. Different letters (a, b, and c) indicate significant differences between treatments (ANOVA followed by multiple comparisons of FDR-corrected LS means; $P < 0.05$). Asterisks indicate overall ANOVA significance (***$P < 0.001$). Raw data, exact biological replicates, and *P* values for all comparisons in this figure are provided in Figshare (*62*).

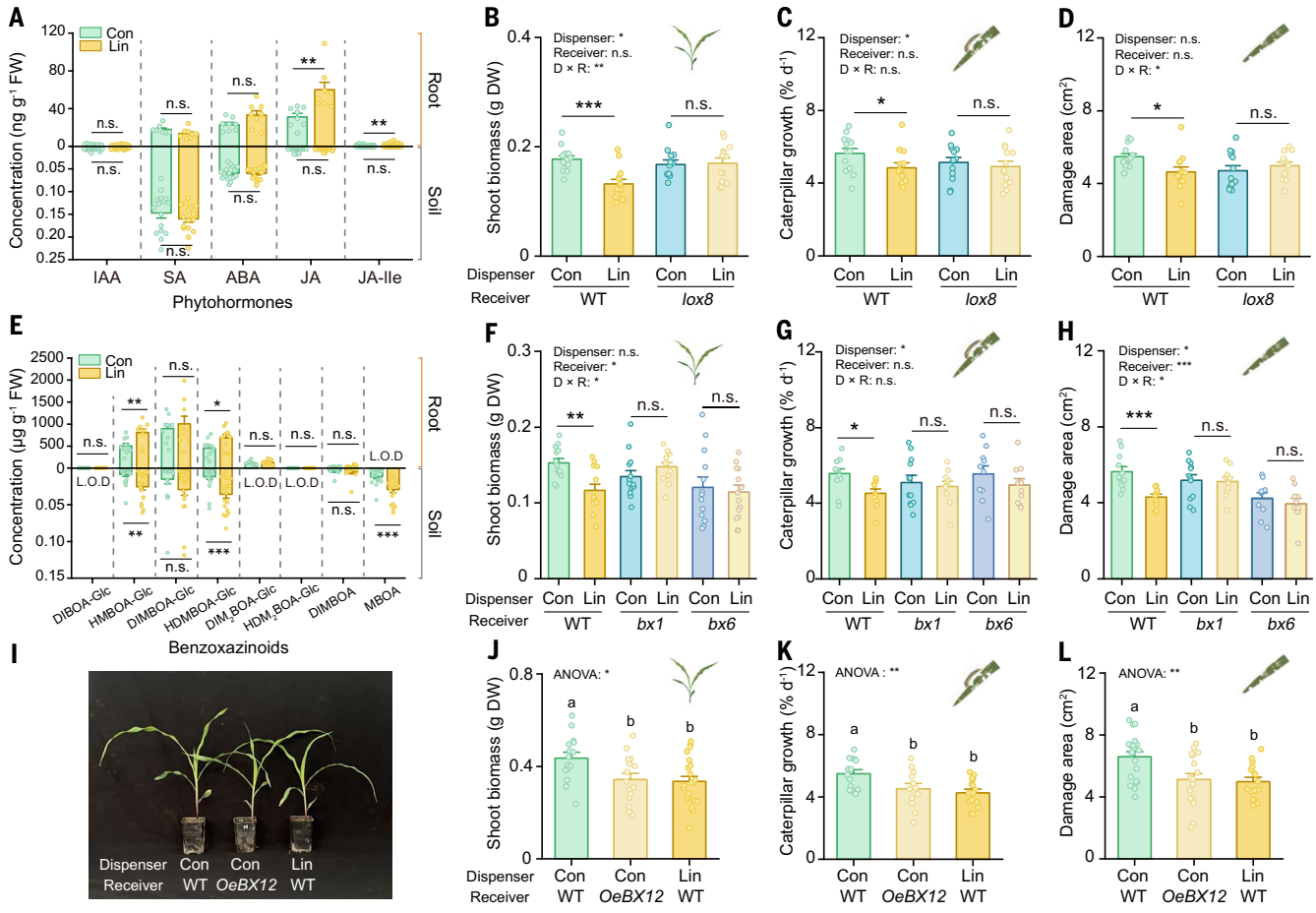

Fig. 3. Linalool triggers plant-soil feedback through JA signaling and BX exudation. (**A**) Concentrations of IAA, SA, ABA, JA, and JA-Ile in the roots or rhizosphere soil of control (Con)– and linalool (Lin)–exposed maize plants (+ SE, $n = 8$ to 16). n.s., not significant. Data points represent individual replicate samples. Asterisks indicate significant differences between treatments (two-sided Student's t test; $^{**}P < 0.01$). ng g^{-1} FW, nanograms per gram fresh weight. (**B** to **D**) Shoot biomass (B), caterpillar weight gain (C), and leaf damage (D) of new maize plants grown in soils previously conditioned by WT and *lox8* plants after Con or Lin exposure (+ SE, $n = 13$). Data points represent individual replicate samples. Asterisks indicate significant differences between treatments (ANOVA followed by pairwise comparisons of FDR-corrected LS means; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). (**E**) Concentrations of BXs in the roots or rhizosphere soil of Con- and Lin-exposed maize plants (+ SE, $n = 9$ to 20). DIBOA-Glc, 2-(2,4-dihydroxy-1,4-benzoxazin-3-one)-β-d-glucopyranose; HMBOA-Glc, 2-(2-hydroxy-7-methoxy-1,4-benzoxazin-3-one)-β-d-glucopyranose; DIMBOA-Glc, 2-(2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one)-β-d-glucopyranose; HDMBOA-Glc, 2-(2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one)-β-d-glucopyranose; DIM_2BOA-Glc, 2-(2,4-dihydroxy-6,7-dimethoxy-l,4-benzoxazin-3-one)-β-d-glucopyranose; HDM_2BOA-Glc, 2-(2-hydroxy-4,7,8-trimethoxy-1,4-benzoxazin-3-one)-β-d-glucopyranose; DIMBOA, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one; MBOA, 6-methoxy-benzoxazolin-2-one. Data points represent individual replicate samples. Asterisks indicate significant differences between treatments (two-sided Student's t test; $^{*}P < 0.05$; **, $P < 0.01$; $^{***}P < 0.001$). µg g^{-1} FW, micrograms per gram fresh weight. L.O.D, limit of detection. (**F** to **H**) Shoot biomass (F), caterpillar weight gain (G), and leaf damage (H) of new maize plants grown in soils previously conditioned by WT, *bx1*, and *bx6* plants after Con or Lin exposure (+ SE, $n = 10$ to 13). Data points represent individual replicate samples. Asterisks indicate significant differences between treatments (ANOVA followed by pairwise comparisons of FDR-corrected LS means; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). (**I**) Growth phenotype of new maize plants grown in soils previously conditioned by WT or *OeBX12* plants after Con or Lin exposure. (**J** to **L**) Shoot biomass (J), caterpillar weight gain (K), and leaf damage (L) of new maize plants growing in soils previously conditioned by WT or *OeBX12* plants after Con or Lin exposure (+ SE, $n = 12$ to 21). Data points represent individual replicate samples. Different letters (a and b) indicate significant differences between treatments (ANOVA followed by multiple comparisons of FDR-corrected LS means; $P < 0.05$). Asterisks indicate overall ANOVA significance ($^{*}P < 0.05$; $^{**}P < 0.01$). Raw data, exact biological replicates, and P values for all comparisons in this figure are provided in Figshare (62).

soil of maize plants after linalool exposure. We observed significant increases in HMBOA-Glc and HDMBOA-Glc in both roots and rhizosphere soil, along with their degradation product, 6-methoxy-benzoxazolin-2-one (MBOA), in the rhizosphere (Fig. 3E). We then used two BX-deficient mutants, *bx1* and *bx6*, as linalool-receiving plants. The *bx1* mutant produces only trace amounts of all BX types, whereas the *bx6* mutant is deficient in HMBOA-Glc, HDMBOA-Glc, and other BX derivatives, but is highly enriched in DIBOA-Glc (26, 27). Both mutants failed to exhibit linalool-induced soil feedback effects on plant growth and resistance (Fig. 3, F to H), highlighting the putative contributions of HMBOA-Glc and HDMBOA-Glc in the observed linalool-mediated feedback.

Because the biosynthetic pathway of HMBOA-Glc is not yet fully elucidated and because MBOA is a breakdown product of BXs and has well-documented effects on plant-soil feedback (25), we focused our attention on HDMBOA-Glc. We overexpressed the HDMBOA-Glc biosynthesis gene *ZmBX12* in B73-329 maize (hereafter *OeBX12*), leading to consistently elevated HDMBOA-Glc levels (27). When *OeBX12* plants were used as receivers, the enhanced herbivore resistance and reduced growth were observed in subsequently grown maize plants, even in the absence of linalool exposure (Fig. 3, I to L). These findings demonstrated that linalool-induced BX exudation, particularly HDMBOA-Glc, is both necessary and sufficient to initiate plant-soil feedback.

Linalool-induced HDMBOA-Glc exudation is regulated by JA signaling

We analyzed the expression of genes involved in the JA and BX pathways in the roots of maize plants after linalool exposure. Linalool treatment significantly up-regulated the expression of key JA biosynthesis genes, including *ZmLOX8*, *ZmAOS*, and *ZmOPR2*, as well as JA signaling genes *ZmMYC2a* and *ZmMYC2b* (*28*–*30*) (Fig. 4A). Concurrently, linalool altered the expression of BX biosynthesis genes, increasing the levels of *ZmBX10/11*, *ZmBX12*, and *ZmBX14*, while decreasing *ZmBX2* and *ZmBX13* expression (Fig. 4A and fig. S14). The expression of JA biosynthesis and signaling genes (*ZmLOX8*, *ZmAOS*, *ZmMYC2a*, and *ZmMYC2b*) was positively correlated with the expression of HDMBOA-Glc biosynthesis genes (*ZmBX10/11*, *ZmBX12*, and *ZmBX14*) (Fig. 4B), suggesting a link between linalool-induced JA signaling and HDMBOA-Glc biosynthesis.

Because *ZmMYC2a* and *ZmMYC2b* have been reported to regulate BX biosynthesis (*28*), we further investigated the connections between JA signaling and HDMBOA-Glc production using *lox8*, *bx1*, and *bx6* mutants. Disruption of JA biosynthesis in *lox8* led to significantly reduced levels of HDMBOA-Glc and other BX derivatives (Fig. 4C). However, knockout or impairment of BX biosynthesis (*bx1* and *bx6*) did not affect linalool-induced JA and JA-Ile levels (Fig. 4, D and E). These results suggest that linalool induces HDMBOA-Glc biosynthesis and exudation primarily through the JA signaling pathway.

Linalool triggers plant-soil feedback through soil microbiota

Rhizosphere-associated microbes respond to hormonal changes in plant roots (*31*) and play an important role in mediating plant-soil feedback (*32*, *33*). We therefore hypothesized that linalool might trigger the feedback by altering the composition and activities of soil microbiota. To test this hypothesis, we grew linalool-exposed maize plants in sterilized bulk soil, and we also reintroduced microbial communities into the sterilized soils by adding suspensions from natural soils conditioned by either control- or linalool-exposed plants. In sterilized soils, the feedback effects on plant growth and resistance were absent. However, these effects were fully restored when microbial suspensions from linalool-treated soils were added (Fig. 5, A to C). These results suggest that soil microbiota are essential for linalool-triggered plant-soil feedback.

Identification of rhizosphere-associated bacteria involved in dense planting–induced soil feedback

Next, we compared the bacterial operational taxonomic unit (OTUs) between control and linalool treatments and found that 111 OTUs were enriched and 129 depleted by linalool exposure (Fig. 5I and data S1). To identify specific bacterial taxa associated with these OTUs, we cultured bacteria from the rhizosphere of both control- and linalool-treated plants, focusing on those sharing 97% 16*S* ribosomal RNA (rRNA) gene similarity

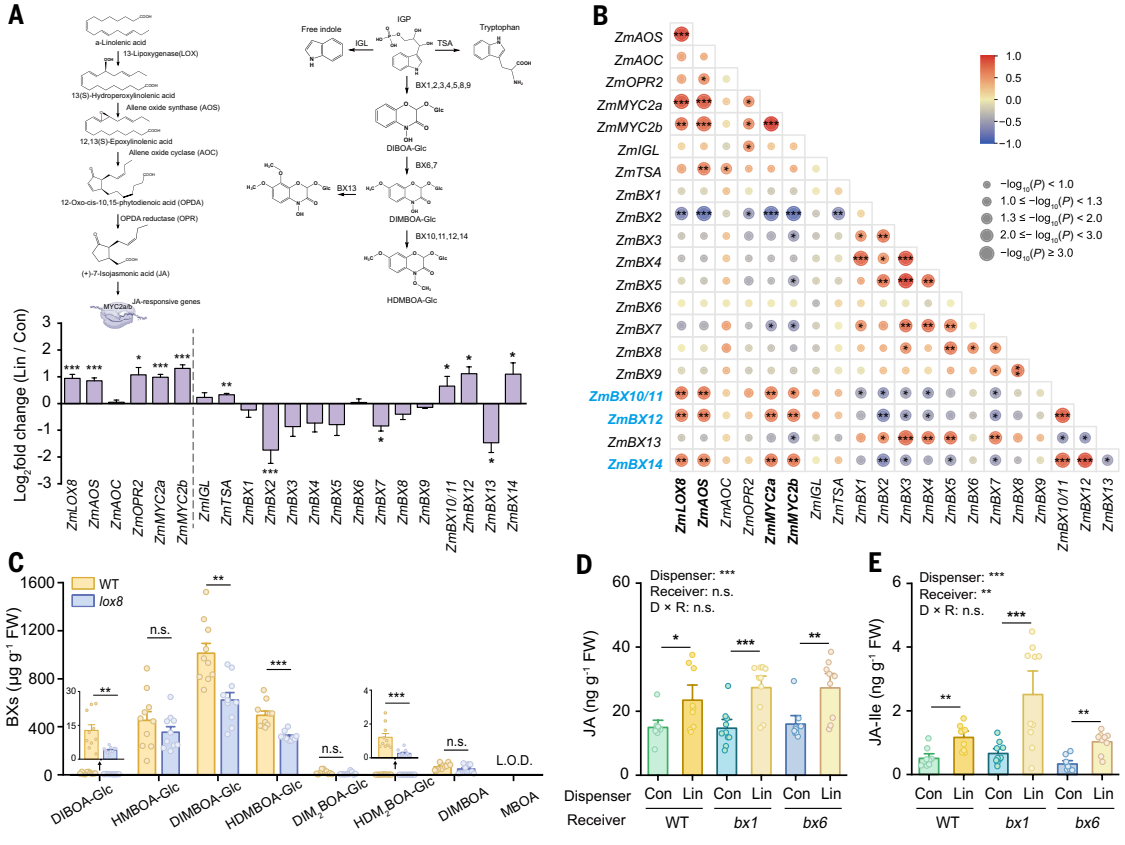

Fig. 4. Linalool-induced HDMBOA-Glc exudation is regulated by JA signaling. (**A**) Log_2 fold changes in the expression of JA and BX biosynthesis genes in maize roots after control (Con) or linalool (Lin) exposure (+ SE, $n = 6$ to 8). Asterisks indicate significant differences between treatments (two-sided Student's *t* test; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). Full datasets are available in fig. S14. Certain components of this figure were generated using BioRender software. (**B**) Pearson correlations between the expression of JA biosynthesis genes and BX biosynthesis genes. Asterisks indicate statistically significant correlations ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$). Genes involved in HDMBOA-Glc biosynthesis are highlighted in bold blue. Genes involved in JA signaling are highlighted in bold black. (**C**) BX content in the roots of WT plants and *lox8* mutants (+ SE, $n = 10$). Data points represent individual replicate samples. Asterisks indicate significant differences between plant genotypes (two-sided Student's *t* test; $**P < 0.01$; $***P < 0.001$). (**D** and **E**) JA and JA-Ile concentrations in the roots of WT plants and *bx1* and *bx6* mutants after Con or Lin exposure (+ SE, $n = 9$ to 10). Data points represent individual replicate samples. Asterisks indicate significant differences between treatments (ANOVA followed by pairwise comparisons of FDR-corrected LS means; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). Raw data, exact biological replicates, and *P* values for all comparisons in this figure are provided in Figshare (*62*).

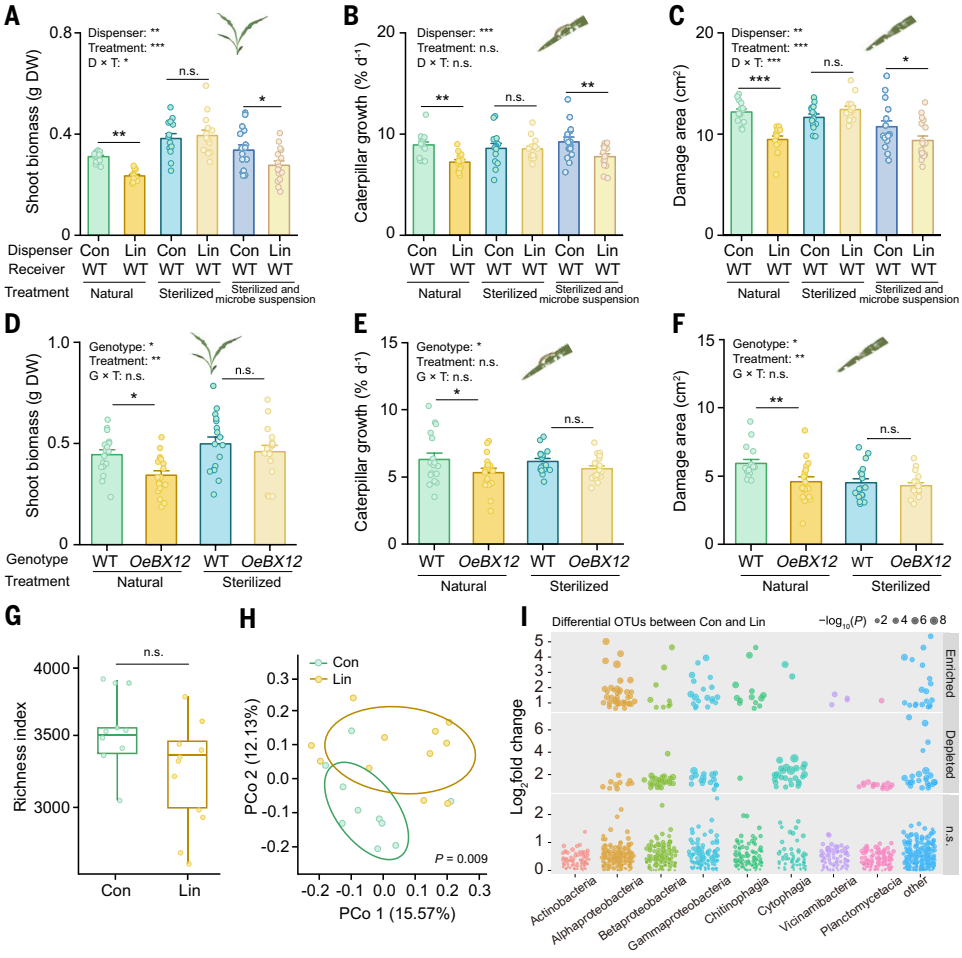

Fig. 5. Soil bacteria mediate linalool-triggered plant-soil feedback. (**A** to **C**) Shoot biomass (A), caterpillar weight gain (B), and leaf damage (C) of maize plants growing in soils previously conditioned by control (Con)– or linalool (Lin)–exposed WT plants (+ SE, $n = 14$ to 18). Soils were either left untreated (natural), x-ray sterilized, or x-ray sterilized and complemented with microbe suspensions from the respective natural soils. Data points represent individual replicate samples. Asterisks indicate significant differences between treatments (ANOVA followed by pairwise comparisons of FDR-corrected LS means; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). (**D** to **F**) Shoot biomass (D), caterpillar weight gain (E), and leaf damage (F) of maize plants grown in soils previously conditioned by WT or *OeBX12* plants (+ SE, $n = 17$ to 18). Soils were either left untreated (natural) or x-ray sterilized. Data points represent individual replicate samples. Asterisks indicate significant differences between treatments (ANOVA followed by pairwise comparisons of FDR-corrected LS means; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). (**G**) Richness index of bacterial communities in the rhizosphere of Con- and Lin-exposed maize plants ($n = 10$ to 11). The horizontal bars within boxes represent medians. The tops and bottoms of boxes represent the 75th and 25th percentiles, respectively. Data points represent individual replicate samples. (**H**) Principle coordinates analysis (PCoA) of the rhizosphere bacterial communities of Con- or Lin-exposed maize plants (PERMANOVA by Adonis). Data points represent individual replicate samples. (**I**) Differential bacteria OTUs between Con- or Lin-exposed maize plants. Each dot represents a single OTU. OTUs enriched or depleted in the soil of Lin-exposed plants were shown (edgeR; FDR-adjusted $P < 0.1$). Raw data, exact biological replicates, and *P* values for all comparisons in this figure are provided in Figshare (*62*).

with detected OTUs. In total, we isolated 94 strains that were significantly enriched in the rhizosphere of linalool-exposed plants (Fig. 6A and data S2).

We then assessed the growth of these 94 strains in minimal media supplemented with microbe-free soil extracts derived from control- or linalool-exposed plants, which retained soluble compounds. Growth assays revealed that 13 strains showed significantly enhanced growth, whereas one strain exhibited suppressed growth in response to linalool-treated soil extracts (Fig. 6A and data S3). To test whether these effects were mediated by HDMBOA-Glc, we measured HDMBOA-Glc levels in the same soil extracts and analyzed their correlation with bacterial growth (data S3). Among the 14 responsive strains, 13 showed a positive correlation between their growth and HDMBOA-Glc concentration, and one strain (L74) did not (Fig. 6A, pie charts; and data S3). We then tested the direct effects of synthetic HDMBOA-Glc on these 14 strains and one negative control strain (L2), previously unaffected by linalool. HDMBOA-Glc stimulated the growth of 12 out of 13 enriched strains but inhibited L9 and L74 (Fig. 6B and fig. S15). The negative control L2 remained unaffected. These results establish a functional link between the root exudate HDMBOA-Glc and growth modulation of specific bacterial taxa enriched in the rhizosphere of linalool-exposed plants.

Next, we complemented soils previously conditioned at 50 plants m^{-2} with these individual bacterial strains and assessed their effects on newly planted maize. Inoculation with HDMBOA-Glc-enriched strains, except for L57 and L91, significantly reduced plant growth, mimicking the phenotypic responses observed in soils conditioned at 200 plants m^{-2} (Fig. 6C). Similarly, inoculation with the enriched strains, except for L57, L78, and L91, significantly enhanced plant resistance (Fig. 6, D and E). By contrast, inoculation with L9, which was depleted by HDMBOA-Glc treatment, promoted plant growth but suppressed resistance in plants grown in soils conditioned at 50 plants m^{-2}. Inoculation with L74 (also depleted strain) or L2 (unaffected strain) had no significant impact on plant growth or defense (Fig. 6, C and E). These findings demonstrate that HDMBOA-Glc–responsive bacterial taxa play an essential role in conferring plant-soil feedback triggered by high planting density and linalool signaling.

Linalool-triggered plant-soil feedback depends on SA signaling

We analyzed the phytohormones, metabolites, and gene expression in new maize plants grown in soils previously conditioned by either control- or linalool-exposed plants. Soils from linalool-exposed plants did not affect levels of defensive metabolites such as BXs or flavonoids but did lead to a reduction in primary metabolites (Fig. 7A and fig. S16). In terms of phytohormones, soils from linalool-exposed plants significantly enhanced SA levels in both the leaves and roots, accompanied by the up-regulation of SA-related signaling genes, such as *ZmWRKY28* and *ZmTGA1* (Fig. 7A and figs. S16 to S18). Additional signaling genes, including *ZmMDL*, *ZmPAL*, *ZmNPR1*, and *ZmNPR3*, were up-regulated in either roots or leaves (Fig. 7A and fig. S17). Conversely, the JA precursor 12-oxo-phytodienoic acid (OPDA) and the JA biosynthesis gene *ZmAOS* were down-regulated in the leaves and roots. IAA and its biosynthesis gene *ZmYucca2* were also specifically down-regulated in the leaves of plants grown in soils from linalool-exposed plants. Defense marker genes were up-regulated in either roots or leaves (Fig. 7A and fig. S17). These findings demonstrate that soils conditioned by linalool-exposed plants induce substantial metabolic and defense reprogramming in new maize plants.

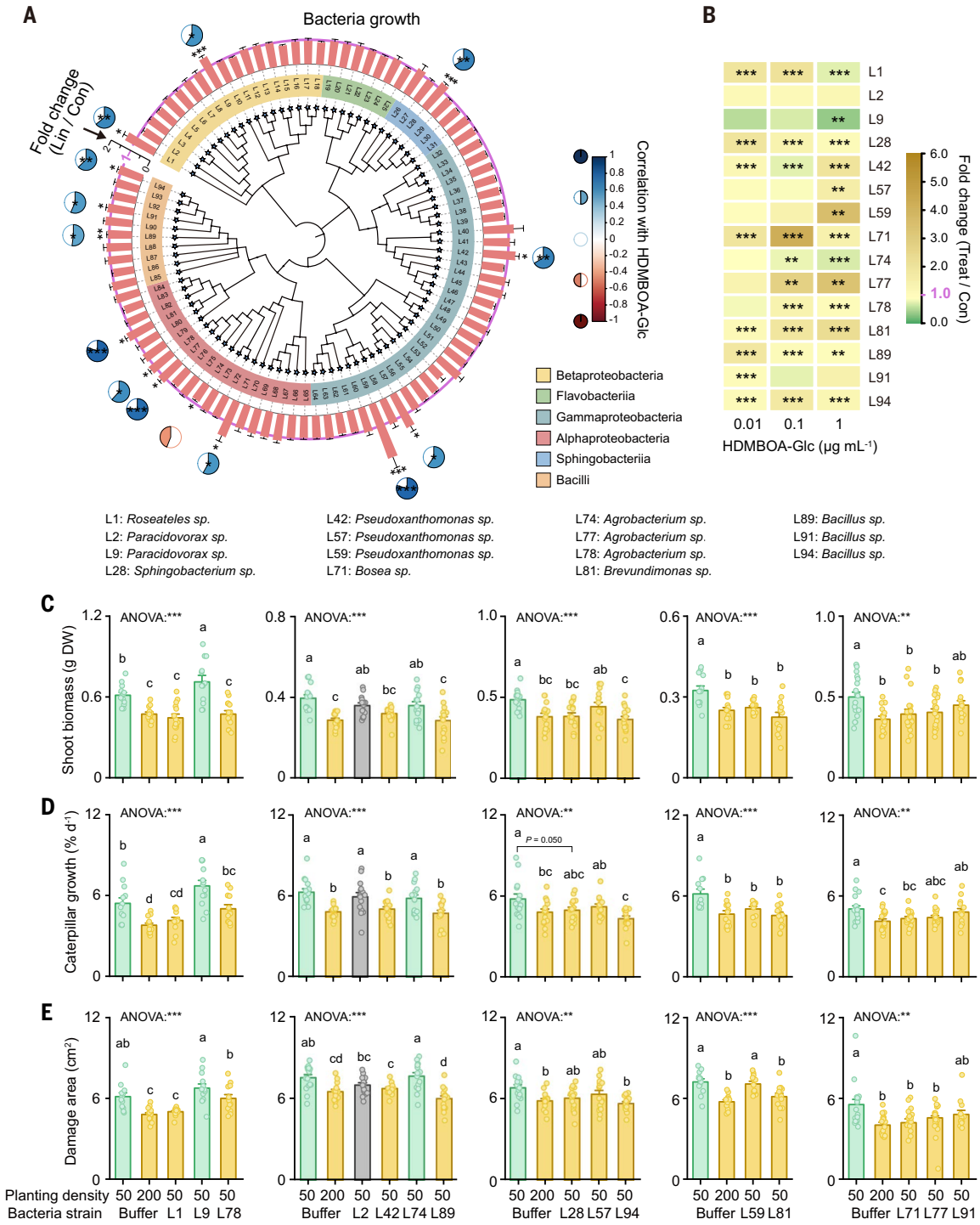

Fig. 6. HDMBOA-Glc–activated bacterial taxa contribute to linalool-triggered plant-soil feedback. (**A**) Phylogenetic trees and growth of 94 bacterial strains isolated from the rhizosphere of maize plants after linalool exposure ($n = 8$). The innermost circle shows the phylogenetic tree of the 94 bacterial strains, with different colors representing various classes. The second circle (pink bars) shows the fold change in growth of the 94 strains when cultivated in soil extracts from control (Con)– or linalool (Lin)–treated plants. The outermost circle (pie charts) indicates the Pearson correlation between bacterial growth and HDMBOA-Glc concentrations in soil extracts from control- or linalool-treated plants. Fold-change values <1.0 indicate reduced growth, while values >1.0 indicate enhanced growth. Asterisks indicate significant correlations (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). For full datasets, we refer to Data S3. (**B**) Heatmaps showing the growth of 15 HDMBOA-Glc–enriched, –depleted, or unaffected bacterial strains after synthetic HDMBOA-Glc treatment. Color intensity indicates the fold changes in bacterial growth upon synthetic HDMBOA-Glc treatment relative to control treatment ($n = 6$ to 8). Fold-change values <1.0 indicate reduced growth, while values >1.0 indicate enhanced growth (full datasets are available in fig. S15). (**C** to **E**) Shoot biomass (C), caterpillar weight gain (D), and leaf damage (E) of wild-type maize plants grown in soils conditioned at 50 plants m^{-2}. These soils were inoculated with HDMBOA-Glc–responsive bacterial strains (+ SE, $n = 12$ to 18). Data points represent individual replicate samples. Different letters indicate significant differences between treatments (ANOVA followed by multiple comparisons of FDR-corrected LS means, $P < 0.05$). Asterisks indicate overall ANOVA significance (**$P < 0.01$; ***$P < 0.001$). Raw data, exact biological replicates, and P values for all comparisons in this figure are provided in Figshare (*62*).

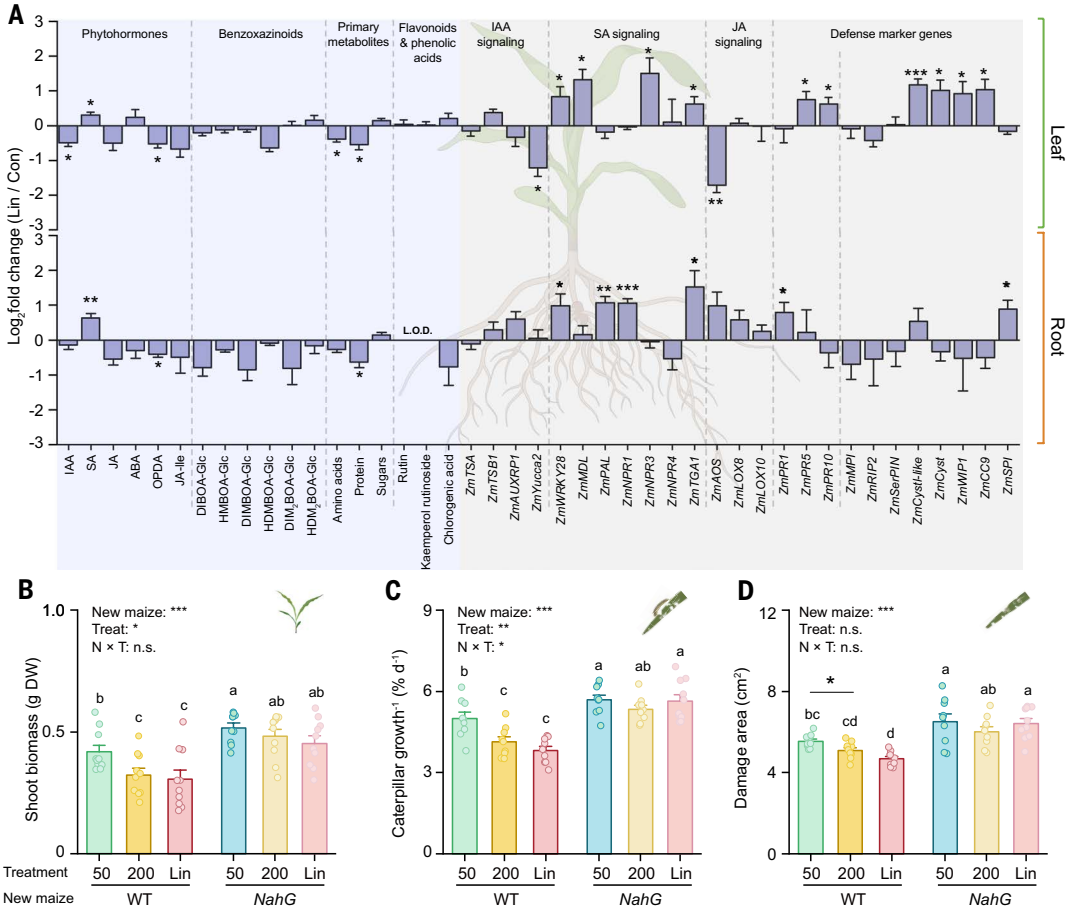

Fig. 7. SA signaling in new maize plants is essential for linalool-triggered plant-soil feedback. (**A**) Log_2 fold changes in phytohormones, metabolites, and gene expression in the leaf and roots of maize plants grown in soils previously conditioned by control (Con)– or linalool (Lin)–exposed receiver plants ($n = 6$ to 10) (full datasets are available in figs. S16 and S17). Certain components of this figure were generated using BioRender software. (**B** to **D**) Shoot biomass (B), caterpillar weight gain (C), and leaf damage (D) of WT and *NahG* overexpression plants grown in soils previously conditioned at 50 or 200 plants m^{-2} or in soils previously conditioned by Lin-exposed plants (+ SE, $n = 9$ to 10). Data points represent individual replicate samples. Different letters indicate significant differences between treatments (ANOVA followed by multiple comparisons of FDR-corrected LS means; $P < 0.05$). Asterisks indicate overall ANOVA significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Pairwise comparisons between selected groups were performed using two-sided Student's *t* test (*$P < 0.05$). Raw data, exact biological replicates, and *P* values for all comparisons in this figure are provided in Figshare (*62*).

Because the increase in SA levels and signaling was the most pronounced in new maize plants and because SA is known to play a key role in the growth and defense trade-off (*34*, *35*), we hypothesized that these altered SA levels might drive the observed shifts in growth and defense in new maize plants grown in soils conditioned by high-density plants. We thus grew the SA-deficient *NahG* lines in soils previously conditioned at 50 or 200 plants m^{-2} (*36*), as well as in soils conditioned by linalool-exposed plants. Our results showed that the feedback effects triggered by planting density on plant growth and herbivore resistance were abolished in the *NahG* lines (Fig. 7, B to D), confirming that SA signaling is required for the dense planting–mediated plant-soil feedback effects on growth and defense.

Discussion

Unlike most maize volatiles, which are specifically induced by biotic or abiotic stress (*37*), linalool is constitutively released, making it a logical candidate for signaling under high-density conditions. Herbivory-induced green leaf volatiles can trigger plant-soil feedback, affecting plant growth and resistance (*38*); our study, however, found that green leaf volatiles were not detectable under high–planting density conditions. Moreover, they induced distinct growth and resistance patterns compared with those observed for linalool (*38*), suggesting that these volatiles are unlikely to play a major role in dense planting–induced plant-soil feedback. However, we cannot completely rule out the potential contribution of other undetected volatiles in our experimental system.

Although linalool stimulates JA biosynthesis in roots, it does not increase JA levels in the rhizosphere, suggesting that JA exudation is an active process not directly regulated by linalool. Disruption of JA biosynthesis resulted in significantly reduced levels of HDMBOA-Glc and other BXs, whereas BX biosynthesis mutants did not affect JA levels. This directional dependency indicates that linalool-induced JA signaling acts upstream of BX biosynthesis and exudation. Supporting this, we observed a positive correlation between the expression of JA biosynthesis and signaling genes and HDMBOA-Glc biosynthesis genes. Furthermore, the JA signaling genes *ZmMYC2a* and *ZmMYC2b* have been shown to directly bind to the promoters of BX biosynthesis genes, transactivating their expression and promoting the production of HDMBOA-Glc and other BX derivatives (*28*). These findings support a model in which JA signaling, mediated by MYC2 transcription factors, orchestrates linalool-triggered BX biosynthesis and root exudation, thereby modulating interactions with the rhizosphere microbiome (fig. S18).

Although linalool has been reported to possess antibacterial properties (*39*), our findings suggest that linalool does not act directly on soil microbes but rather influences them indirectly through physiological

changes in the roots of neighboring plants. Only 3 days of high-density treatment were sufficient to trigger plant-soil feedback, implying that rhizosphere microbial communities belowground can respond rapidly to volatile cues aboveground. These microbial compositional shifts appear to persist long enough to alter the growth and defense phenotypes of subsequently grown plants, highlighting the speed and stability of volatile-mediated plant-microbe interactions in dense cropping systems.

Microbial communities are highly responsive to environmental context, and changes in the presence or abundance of specific taxa can reflect localized conditions such as plant density (*40*). Although microbial populations are dynamic, their functional roles can remain sufficiently consistent to serve as indicators of environmental change. In this context, microbiome members may serve not only as mediators of plant-soil feedback but also as conveyors of ecological information (*41*). Our findings support the hypothesis that plants may use microbial signals as indicators of local plant population density, allowing them to fine-tune their growth and defense responses accordingly (*42*).

To begin to understand the importance of the rhizosphere microbial taxa, we used 97% identity of the 16*S* amplicon sequences to link isolated bacterial strains to OTUs. This identity threshold has its limitations to fully capture species- or function-level variation because it may group together taxa with genetic and functional divergence, including members of different genera (*43*). A more comprehensive approach would involve whole-genome sequencing of both the microbiome and the cultured isolates to capture their full functional potential at the species or even strain level. In addition, future studies should explore the role of rhizosphere-associated fungi and more-complex synthetic microbial consortia, including both bacterial and fungal members (*44*).

We observed similar growth and resistance phenotypes in *bx6* and *OeBX12* lines under control conditions, despite their markedly different HDMBOA-Glc levels (*27*). This suggests that additional BX intermediates or regulatory factors may contribute to the observed phenotypes. HDMBOA-Glc and its degradation product, MBOA, are known to have antimicrobial or growth-promoting properties (*45*, *46*), which may explain their ability to enrich and deplete specific soil bacteria. MBOA accumulated in rhizosphere soils after linalool exposure, which is consistent with findings that MBOA triggers similar feedback effects, enhancing herbivore resistance and reducing growth (*25*). Thus, linalool-driven root HDMBOA-Glc exudation modulates the composition of the rhizosphere microbiota, driving the observed plant-soil feedback (fig. S18).

Although JA is classically associated with antiherbivore defenses (*47*), our data showed reduced JA signaling in the plants subsequently planted, suggesting that JA may not directly mediate the observed resistance at this stage. By contrast, SA signaling proved essential for the expression of enhanced resistance, aligning with previous findings in maize (*36*, *48*, *49*). The concurrent up-regulation of other defense-related genes implies that additional signaling pathways may also contribute.

Linalool-induced JA signaling occurs during the conditioning phase, where it promotes the exudation of BXs and shapes the rhizosphere microbiome. In the response phase, the subsequently grown plants rely primarily on SA signaling to express enhanced defense phenotypes (fig. S18). This suggests a temporal decoupling between signal perception (JA-driven) and defense expression (SA-driven), with each hormone acting at distinct stages of the feedback loop (*50*, *51*). In a densely planted field, such hormonal dynamics may operate within a single generation through continuous cycles of linalool perception and response. Plants might sense volatiles from neighbors, adjust their exudate profiles, and modulate their associated microbiota accordingly. This could enable real-time reinforcement of systemic resistance through microbiome-mediated signaling. Coordinated JA and SA signaling, both spatially and temporally, may thus be a key feature of plant adaptation to dense cropping systems. Disentangling intergenerational versus intragenerational roles of these pathways represents an exciting avenue for future research. In particular, elucidating how maize leaves perceive and transduce linalool signals will be essential for resolving this regulatory complexity.

The physiological changes of maize plants in the response phase offer insights into the trade-offs involved in volatile-mediated plant-soil feedback. First, increased herbivore resistance in subsequently grown maize plants correlates with elevated expression of defense-related genes (*52*), whereas reduced plant growth was associated with lower levels of free amino acids and soluble proteins. Second, we observed a classic JA/SA signaling trade-off (*53*), with decreased JA biosynthesis and increased SA biosynthesis. This reprogramming of primary metabolism toward enhanced defense suggests that maize plants prioritize defense over growth under high-density conditions, likely as an adaptive response to biotic pressures from neighboring plants (*54*, *55*). Although this trade-off limits biomass accumulation, the enhanced resistance to herbivores and pathogens may outweigh the costs, particularly in environments with high biotic stress. Furthermore, the reduction in growth and increase in defense could be beneficial in agricultural systems, potentially lowering pesticide inputs and reducing the risk of lodging to some extent. Although individual plant yield is reduced in dense planting systems, overall crop yield is often higher compared with that observed for low-density planting (*2*, *8*, *56*). This highlights the importance of balancing growth and defense in optimizing plant performance under dense planting conditions.

Informed by these findings, we propose to use linalool as a breeding target for regionally adapted maize varieties. In regions with high pathogen or pest pressure, breeding or engineering crop varieties with enhanced linalool emission or increased BX biosynthesis could optimize plant defenses in dense planting systems. Conversely, in areas with lower biotic stress, reducing linalool biosynthesis or improving planting designs to enhance air circulation could prevent linalool accumulation, thereby increasing maize productivity in dense cultivation. Substantial field research will be necessary to translate these strategies into practice. It is important to note that the field experiments presented in this study were not intended to directly test the linalool-mediated feedback mechanism but were designed to examine whether planting density influences plant growth and resistance under natural conditions. Rigorous field validation, such as the application of synthetic linalool under controlled field conditions and the assessment of downstream responses, will be essential and represents a promising direction for future research. Our study provides new insights into plant plasticity in response to environmental cues (fig. S18), highlighting the importance of volatile-mediated plant-soil feedback as a promising strategy for sustainable agricultural intensification and global food security.

Materials and methods

Plants

In this study, we used maize (*Zea mays*), barley (*Hordeum vulgare*, cultivar Eunova), and ryegrass (*Lolium perenne*, cultivar Pr521). The maize genotypes included the linalool-deficient mutant *tps2* and its wild-type (WT) B73; two BX-deficient mutants, *bx1* and *bx6*, and their WT W22; the HDMBOA-Glc overexpression line *OeBX12* and its WT B73-329; the JA-deficient mutant *lox8* and its WT W22; and the SA-deficient line *NahG* and its WT KN5585. The genotypes *bx1*, *bx2*, and *NahG* have been previously described and characterized (*27*, *36*).

The *OeBX12* line was specifically created for this study. Briefly, the open reading frame of the *ZmBX12* gene was cloned from CML322 inbred line and inserted into a *pCombia3300-ZmUbi*-derived binary vector. The constructed vectors were transformed into the recipient line B73-329 via *Agrobacterium tumefaciens*-mediated transformation. Transformed lines exhibiting the strongest HDMBOA-Glc accumulation phenotypes were selected for propagation. Homozygous lines were used for experiments alongside the WT line B73-329. Overexpression of *ZmBX12* was confirmed by quantitative real-time polymerase chain reaction (QRT-PCR) as described in the "Gene expression analysis" section, and HDMBOA-Glc over-accumulation in the roots was determined as described in the "Secondary metabolite analysis" section.

The *lox8* mutant was acquired from the Maize Genetics Cooperation Stock Center at the University of Illinois at Urbana-Champaign (Maize COOP; http://maizecoop.cropsci.uiuc.edu) as a mixture of WT, heterozygous, and homozygous seeds. Homozygous individuals were genotyped using gene-specific primers and further selfed for experiments. The effects of the *lox8* mutation on jasmonate biosynthesis were confirmed by quantifying the levels of JA, and JA-Ile using the protocol described in the "Phytohormone analysis" section.

The *tps2* mutant was obtained from the Maize EMS Database (maizeEMSDB; http://maizeems.qlnu.edu.cn). The *tps2* mutant harbors a single C-to-T mutation in the first exon, resulting in a premature termination codon of the *tps2* gene (fig. S11A). The effects of the *tps2* mutation on linalool biosynthesis were confirmed by profiling volatile compounds using the protocol described in the "Volatile profiling" section (fig. S11B).

Field survey

To explore whether planting density affects plant growth and defense through plant-plant interactions, a herbivory survey was conducted across four independent maize fields in Ledong, Hainan Province, China, during the winter of 2024. The fields surveyed were as follows: Field I (18°33′50″N, 108°55′13″E; area: 660 m^2), Field II (18°33′54″N, 108°55′09″E; area: 600 m^2), Field III (18°34′1″N, 108°54′57″E; area: 2100 m^2), and Field IV (18°34′10″N, 108°54′56″E; area: 2200 m^2). In China, the optimal planting density for maize is estimated to range between approximately 71,000 to 86,000 plants ha^{-1} (*3*). Accordingly, Fields I and II were planted with the maize cultivar Taiyanghua6 at a planting density of ~60,000 plants ha^{-1}, which falls below the standard agronomic recommendation. In contrast, Fields III and IV were planted at a high density of around 120,000 plants ha^{-1}, exceeding current best practices and reflecting emerging strategies for ultra-dense planting aimed at maximizing yield. To evaluate plant-plant interactions, each field was divided into inner sections and outer edges, and variations in growth and herbivory were assessed across these regions (Fig. 1A). For each area, four to six blocks were randomly selected, providing four to six biological replicates per treatment. At the tasseling stage, 100 to 256 plants (depending on field size) were randomly selected from each block to quantify herbivory damage. Herbivore damage was visually evaluated using a standardized leaf damage rating scale and quantified using a weighted attack intensity, following the FAO-recommended method described by Kalqutny *et al.* (*57*). Additionally, the plant height, shoot biomass, 1000-grain weight and grain yield per ear were measured in these four fields.

Soil conditioning by different planting densities

To investigate how planting density influences plant growth and defense through plant-soil feedback, we collected natural soil from a field in Hangzhou, China (30.3076 N, 120.0749 E). This bulk soil was sieved through a 4.75-mm mesh and air-dried for 48 hours. The soil was then placed into 400 ml or 12-liter sterile pots. Uniformly pre-germinated maize B73 seeds were sown in these pots. The maize plants were grown in a greenhouse maintained at 28°C ± 2°C with 55% relative humidity, a 14:10 hour light/dark cycle, and a light intensity of 350 μmol m^{-2} s^{-1}. The environment was complemented by continuous vertical airflow at 0.5 ms^{-1}. Plants were watered as needed. At the four-leaf stage, approximately 12 days post-planting, maize plants were repositioned at varying planting densities—50, 100, 150 and 200 plants m^{-2}—for a period of 3 days. (Fig. 1C). These were arranged within a well-ventilated transparent plastic sheet with an open-air top for 8 hours per day (9:00 a.m. to 5:00 p.m.). Three days after this density treatment, the plants were removed, and the soil was collected and sieved to be used for growing new plants. New maize plants were then planted into these conditioned soils and spaced to prevent direct density effects on their growth and defense. This experimental design ensured that any observed differences in the growth or defense of new plants were attributed to plant-soil feedback rather than direct root-root interactions or immediate density effects. The planting density were characterized by the levels of photosynthetically active radiation (PAR) and the red-to-far-red (R/FR) light ratio beneath the plant canopy. PAR, quantum efficiency (Eq), red light ratio (Rratio), and photosynthetic photon flux density (PPFD) were detected using spectrometer PLA-20 (EVERFINE Corporation, China). R/FR = [Eq×Rratio-PPFD (FR)]/ PPFD (FR).

Feedback experiment

Uniformly pre-germinated B73 seeds were sown individually in soils previously conditioned by maize plants grown at different planting densities. The new maize plants were spaced and cared for as described above. Shoot root biomass and plant resistance were assessed for these new plants. Specifically, shoot biomass was recorded 14 days after planting in 400-ml pots. Shoots and roots were harvested, oven-dried at 60°C for 2 days, and weighed to determine biomass. Details on plant resistance evaluation are provided in the "Plant resistance evaluation" section.

To determine whether plant density-triggered feedback effects are genotype-specific, three additional maize cultivars, B104, W22, and KN5585, were planted in soils previously conditioned by their respective cultivar at 50 or 200 plants m^{-2}. Plant growth and resistance for B104, W22, and KN5585 were assessed as described above.

To investigate whether plant density-triggered feedback occurs between different plant species, maize plants were cultivated alongside 10-day-old ryegrass (15 to 20 plants per pot) or barley (15 to 20 plants per pot), and the feedback effects in maize-conditioned soils were assessed as described above (fig. S6A).

To determine whether the observed feedback effects are specific to maize, we also grew ryegrass or barley in soils previously conditioned by maize at planting densities of 50 or 200 plants m^{-2} (fig. S7A). Additionally, ryegrass and barley were used to condition soils at low and high planting densities, and their subsequent growth and herbivore resistance were evaluated in their own conditioned soils (fig. S8A).

Plant resistance evaluation

Growth and damage of leaf herbivores: To assess the impact of planting density-triggered plant-soil feedback on maize resistance to leaf herbivores, we evaluated the growth and damage caused by fall armyworm (*Spodoptera frugiperda*) larvae. The larvae were reared on an artificial diet as previously described (*26*). A single starved, pre-weighed second instar larva was placed individually in a cylindrical mesh cage (1-cm height, 2.4-cm diameter). These cages were clipped onto the leaves of individual plants grown in soils conditioned at either 50 or 200 plants m^{-2}. The cage position was adjusted daily to provide sufficient food for the larvae. Larval mass was measured 3 to 5 days after the experiment began. Leaf damage was quantified by scanning the leaves and determining the area removed using Digimizer software.

Infection ability of root herbivores: To evaluate the effect of planting density-triggered plant-soil feedback on maize resistance to root herbivores, we assessed the infectivity of the root-knot nematode (*Meloidogyne incognita*). Nematode eggs were collected from infected tomato roots using a syringe needle and incubated at 28°C for 2 to 4 days to hatch J2 juveniles. Fourteen-day-old maize seedlings, grown in soils conditioned at either 50 or 200 plants m^{-2}, were inoculated with approximately 400 J2s. Fourteen days after infection, the number of galls and root biomass were recorded as previously described (*58*).

Infectivity of fungal pathogens: To determine the impact of planting density-triggered plant-soil feedback on maize resistance to plant pathogens, we evaluated the infectivity of *Exserohilum turcicum*, the causative agent of northern corn leaf blight (NCLB), which is a devastating prevalent disease in maize field. *E. turcicum* was incubated at 24°C for 2 days in Potato Dextrose Broth (PDB) with shaking (180 rpm). The fungus was harvested by centrifugation (5000 g, 10 min), resuspended

in distilled water, and filtered through cotton percale to remove mycelium, collecting the spores. Spores were then suspended to a concentration of 1×10^4 ml^{-1} using a hemocytometer. Maize plants were initially grown in 1-liter pots to condition soil at either 50 or 200 plants m^{-2}, as described above. The conditioned soils were then pooled into 12-liter pots, and new maize plants were individually transplanted in these larger pots. Forty-five days after planting, spore suspensions were evenly sprayed on the new plants. Twenty days after inoculation, lesion areas were excised from the leaves, adhered to A4 paper, scanned, and the lesion area was quantified using Digimizer software.

Virulence of rice black-streaked dwarf virus: To investigate the effect of planting density-triggered plant-soil feedback on maize resistance to phytovirus, we assessed the virulence of rice black-streaked dwarf virus (RBSDV), an arbovirus transmitted by planthoppers (*Laodelphax striatellus*) that causes maize rough dwarf (MRD) disease. New maize plants grown in soils conditioned at 50 or 200 plants m^{-2} were randomly arranged in a 0.5 m^3 insect-proof tent. At the two-leaf stage, these plants were artificially inoculated with RBSDV by allowing viruliferous planthoppers to feed on them for 3 days. The tent was then removed, and the infected plants were rearranged in the greenhouse. MRD symptoms were evaluated 80 days after inoculation. Disease severity index (DSI) calculation and scoring criteria were conducted as previously described (*59*). Virus concentration was also determined by QRT-PCR using primers RB-CP-216-F (CTTCATGATAGACTGCAAACCGTA) and RB-CP-359-R (ACAAATTCTTCACTTAGGTCGTT).

Volatile profiling

To assess maize volatile dynamics at different planting densities in situ, we employed the solid phase microextraction (SPME) method to profile leaf volatiles. A SPME fiber was positioned above the canopies of 14-day-old maize plants grown at 50, 100, 150 or 200 plants m^{-2}. The fiber absorbed volatiles for 60 min. The incubated fibers were then immediately analyzed using GC-MS (Agilent 7820A GC) according to previously established settings and protocols (*18*).

To investigate linalool emission from maize, ryegrass, barley, and *tps2* mutant plants, volatiles were collected using a dynamic headspace sampling system and Super-Q traps. Detailed information on the volatile sampling system and analysis methods has been previously published (*18*).

Construction and optimization of linalool dispensers

To confirm the effects of linalool in triggering plant-soil feedback, we constructed a dispenser designed to release synthetic linalool, as previously detailed (*18*). To ensure that maize plants were exposed to a physiological dose of synthetic linalool, corresponding to the amounts emitted by maize plants at different planting densities, we placed SPME fibers at varying distances (5 cm, 10 cm, 20 cm) from the dispenser. Additionally, fibers were placed midway between two dispensers positioned 20 cm apart in 0.07 m^2 open-air plastic cylinders (30 cm in diameter, 1 m in height, open at the top) to capture the gradient of emitted linalool (fig. S9C). The volatile compounds adsorbed by the SPME fibers were analyzed as described in the "Volatile profiling" section. We found that at a distance of 5 cm from a single linalool dispenser, the linalool concentration simulated the levels measured under a planting density of 150 plants m^{-2} (fig. S9D).

Soil conditioning via linalool exposure

To simulate the physiological linalool concentrations associated with different maize planting densities, we adjusted the exposure setup (fig. S9D). Maize plants were exposed to a linalool dispenser placed at varying distances: ~5 cm, 10 cm, 20 cm, or between two dispensers spaced 20 cm apart, to replicate the concentrations observed in different planting density conditions (fig. S9E). After 3 days of exposure (6 hours per day), soils from both the control- and linalool-exposed plants were collected and used to grow new maize plants. The growth and defense responses of these plants were then assessed as detailed in the "Feedback experiment" section to evaluate the feedback effects. Our findings indicated that exposure to linalool at a 5-cm distance, which mimics concentrations found under a planting density of 150 plants m^{-2}, effectively triggered plant-soil feedback effects (Fig. 2, B to D, and fig. S9E). Therefore, for all subsequent experiments, maize plants were exposed to a linalool dispenser at a 5-cm distance (hereafter referred to as "Lin").

Soil BX analysis

To determine the BX concentrations in soil, rhizosphere soil surrounding maize roots was carefully collected. Three maize seedlings, along with their adhering rhizosphere soil, were placed into a sealable bag. The bag was gently shaken to avoid damaging the roots while ensuring sufficient rhizosphere soil was collected. The collected soil was then sieved through a 250-μm mesh to remove root residues. The BXs in the sieved soil were extracted and analyzed following the procedure described (*26*).

Phytohormone analysis

For phytohormone analysis in maize leaves and roots, OPDA, JA, JA-Ile, SA, IAA, and ABA were extracted using ethyl acetate with isotopically labeled standards (1 ng for d_6-JA, d_6-JA-Ile, d_4-SA, d_5-IAA and d_6-ABA) and quantified via ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS), following the methods in our previous work (*18*).

For phytohormone analysis in conditioned soil, soil samples were collected and processed as described in the above "Soil BX analysis" section. A 10-g soil sample was thoroughly homogenized in 10 ml of acetone. The mixture was subjected to vigorous vortexing for 5 min, followed by centrifugation at 4000 g for 10 min. The supernatant was collected and further centrifuged at 15,000 g for 10 min. The final supernatant was evaporated to dryness, and the residue was dissolved in ethyl acetate, supplemented with isotopically labeled standards. The supernatant was then evaporated to dryness, redissolved in methanol and analyzed using UPLC-MS/MS (*60*).

Gene expression analysis

QRT-PCR was used to measure gene expression levels in maize leaves and roots. Total RNA was isolated using the Tiangen Plant RNA Purification Kit (TIANGEN, China) according to the manufacturer's instructions. Eight hundred nanograms of each RNA sample was reverse transcribed using the PrimerScript RT Master Mix (Takara, China). The QRT-PCR assays were performed on a LightCycler 96 Instrument (Roche, Switzerland) using the KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems, USA). The maize actin gene (*ZmActin*) was used as an internal reference to normalize cDNA concentrations. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used for QRT-PCR of all tested genes are listed in data S4.

Microbial sterilization and complementation

To investigate the role of soil microbiota in linalool-mediated feedback, bulk field soil was either sterilized by x-ray irradiation [50 kilograys (kGy)] at Shanghai Co-Elit Agricultural Sci Tech Company, China, or left untreated (Natural). The sterilized soil was also complemented with microbe suspensions obtained from soils of control- and linalool-exposed plants. The microbe suspensions were prepared by mixing 400 ml of soil with 400 ml of autoclaved Milli-Q water. The mixture was allowed to stand for 2 hours to let larger soil particles settle. The supernatant was then passed through a 250-μm sieve, followed by two successive 10-μm sieves to retain nematodes and spores of most species of arbuscular mycorrhiza, while allowing the suspended microorganisms to pass through. A volume of 100 ml of these microbial suspensions was used to complement the sterilized soil. This process resulted in six soil treatments: natural control soil, natural linalool-exposed soil, sterilized control soil, sterilized linalool-exposed soil, and sterilized

control and linalool soils complemented with microbial suspensions. Maize plants were planted in these soils and exposed to either control or linalool dispensers as previously described. After exposure, new maize plants were planted into the conditioned soils, and their growth and herbivore resistance were assessed according to the feedback experiment protocol.

Soil bacteria profiling

Rhizosphere soil samples were collected as described previously (*38*). Approximately 200 mg of fine rhizosphere soil was used for DNA extraction with the FastDNA SPIN Kit for Soil (MP Biomedicals, USA), following the manufacturer's instructions. The V4-V5 region of the bacterial 16*S* rRNA gene was amplified using the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 907R (5′-CCGTCAATTCCTTTGAGTTT-3′). PCR products were verified for size and purity by gel electrophoresis, followed by gel purification with the E.Z.N.A. Gel Extraction Kit (Omega, USA) and subsequent DNA quantification. Sequencing libraries were prepared using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, USA) according to the manufacturer's protocols, incorporating index codes for sample identification. Library quality was assessed with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, USA), and sequencing was performed on an Illumina HiSeq PE250 platform (Illumina Nova 6000, USA) by Novogene (Novogene, China). The 16*S* rRNA gene sequences were analyzed using EasyAmplicon, following the approach outlined by Liu *et al.* (*61*). Operational taxonomic units (OTUs) were generated with a similarity threshold of 97% (-otutab, -id 0.97), and taxonomic annotation was performed using USEARCH with the RDP database (-sintax_cutoff 0.6). Bray-Curtis diversity metrics were calculated using EasyAmplicon, with pairwise Anosim tests and permutational multivariate analysis of variance (PERMANOVA) conducted with 999 permutations. The results were visualized through principal coordinates analysis (PCoA) plots. Differential OTU abundance was analyzed for OTUs with a mean relative abundance >0.01% using edgeR, and *P* values were adjusted using the false discovery rate (FDR) with a threshold of 0.1.

Isolation and identification of rhizosphere-associated bacteria

To isolate linalool-responsive bacteria strains, soils from control- and linalool-exposed plants were vortexed in 0.1 M phosphate-buffered saline for 5 min. The resulting suspensions were serially diluted, plated on 9-cm Petri dishes, and cultivated on various media including Nutrient Broth agar (NA), tryptic soy agar (TSA), 1:10 (v/v) TSA, Reasoner's 2A agar (R2A), Luria Bertani (LB) agar, and 1:10 (v/v) LB agar. After 2 to 5 days of incubation, single colonies were isolated by streak plating. Genomic DNA was extracted from the purified bacterial colonies, and the 16*S* rRNA gene was amplified. The PCR products were validated by Sanger sequencing using the 27F (5′-GAGTTTGATCATGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) primers. Bacteria that shared >97% 16*S* rRNA gene sequence similarity with OTUs enriched in the rhizosphere of linalool-treated plants were selected as candidates for subsequent experiments (data S2). We focused on strains enriched under linalool exposure conditions, as these could be experimentally reintroduced into low-density soils to test their functional roles (see "Bacterial complementation experiment" section). In contrast, selectively removing depleted strains from high-density soils to assess their specific contributions remains technically challenging with current methodologies.

Bacterial growth analysis

To evaluate the effect of linalool exposure on the growth of rhizosphere-associated bacteria, soil extracts were collected from both control- and linalool-exposed maize plants. The extracts were prepared by suspending 10 g of conditioned soil in 10 ml of water. Subsequently, the mixture was vortexed at 2000 g for 10 min, followed by centrifugation at 6000 g for another 10 min. Finally, the supernatant was filtered through a 0.22-μm Nalgene filter unit. These filtered extracts were then mixed with culture medium in a 9:1 ratio (v/v). For the bacterial growth, 1 μl of a 0.1 OD pre-cultured bacterial suspension was inoculated into 200 μl of the fresh soil extract mixture in 96-well microtiter plates.

Bacterial growth was determined by measuring absorbance at 600 nm using a Microplate Reader (Biotek, Synergy H1, USA) after 24 hours of incubation (180 rpm, 30°C). To examine the correlation between HDMBOA-Glc concentrations in soil and bacterial growth, we quantified the concentration of HDMBOA-Glc and performed a Pearson correlation analysis to assess the relationship between HDMBOA-Glc levels and bacterial growth.

Direct influence of HDMBOA-Glc on bacterial growth

To assess the direct impact of synthetic HDMBOA-Glc on the growth of rhizosphere-associated bacteria, we quantified bacterial proliferation in soil extracts treated with varying concentrations of HDMBOA-Glc. Soil extracts were collected from field bulk soil with no detectable HDMBOA-Glc. The extracts were prepared as described in the "Bacterial growth analysis" section above. Synthetic HDMBOA-Glc was added to 200 μl of the soil extracts to achieve final concentrations of 0, 0.01, 0.1, and 1 μg ml^{-1}. Subsequently, 1 μl of each precultivated bacteria strains with an OD of 0.1 was inoculated into the 200-μl soil extracts in 96-well microtiter plates. After 24 hours of incubation, 50 μl of the planktonic bacterial suspension was collected, serially diluted, and plated on 9-cm Petri dishes. The plates were incubated for 1 to 3 days, depending on the growth kinetics of the bacterial strains. Phenotypic observations were documented through photographic records of the bacterial colonies. Colony-forming units (CFU) were counted using a Dot Counting Camera (developed by Beijing Pengtuzhumeng Technology Co., Ltd.), and the counts were adjusted according to the dilution factor.

Bacterial complementation experiment

To test whether HDMBOA-Glc-responsive microbes are sufficient to induce growth and defense effects in maize plants, we selected 13 HDMBOA-Glc-enriched strains, 1 depleted strain, and 1 unaffected strain to complement the soils previously conditioned at 50 plants m^{-2}. Each bacterial strain was cultivated in its respective liquid medium for 1 to 2 days in a shaker (180 rpm, 30°C) and harvested by centrifugation (5000 to 12,000 g, 10 min). The harvested bacteria were washed three times with ddH_2O and resuspended in ddH_2O to a final concentration of 1×10^7 CFU ml^{-1}. For the inoculation, 10 ml of each bacterial suspension was added to the conditioned soils. Uniformly pregerminated WT maize seeds were individually sown in these bacteria-complemented soils. Shoot biomass, herbivore resistance, and leaf damage area were quantified as described above.

Primary metabolite analysis

To assess the impact of linalool-triggered plant-soil feedback on primary metabolites, we quantified free amino acids, total sugars, and soluble protein levels in the maize grown in soils conditioned by linalool-exposed plants. Free amino acids were analyzed using the UPLC-MS/MS method (*26*). Total soluble proteins were determined using the Bradford assay, and total sugars were measured with the 3,5-dinitrosalicylic acid (DNS) method. For sugar analysis, 0.5 g of fresh leaf tissue was homogenized in 1 ml of deionized water and 500 μl of 6 M HCl, then sealed and incubated at 90°C for 30 min. After cooling to room temperature, the mixture was neutralized with 500 μl of 6 M NaOH, and the final volume was adjusted to 2 ml with thorough mixing. Following centrifugation at 12,000 rpm for 10 min, 200 μl of the supernatant was collected. This supernatant was then heated to 95°C, after which an equal volume of DNS reagent (Macklin) was added until a red coloration developed. Absorbance was measured at 540 nm using a Microplate Reader (Biotek, Synergy H1, USA).

Secondary metabolite analysis

To assess the impact of linalool-triggered plant-soil feedback on secondary metabolites, we quantified the levels of BXs, flavonoids, and

phenolic acids in maize grown in soils conditioned by linalool-exposed plants following a standardized procedure. Briefly, 50 mg of flash-frozen maize leaf or root samples were extracted with 1 ml of an acidified H_2O/MeOH solution (50:50 v/v, containing 0.1% formic acid). The extracts were analyzed using an Acquity UPLC-MS/MS system equipped with an electrospray ionization source, using the parameters established in our previous work (*26*). Absolute concentrations of BXs, flavonoids and phenolic acids were determined using standard curves generated from pure synthetic compounds.

Feedback experiment with SA-deficient lines

The uniform and pre-germinated *NahG* overexpression lines as well as its WT KN5585 plants were individually sown in soils which were previously conditioned by control- or linalool-exposed B73 plants. These plants were taken care of as described above. The shoot biomass, herbivore resistance and leaf damage of *NahG* overexpression and KN5585 plants were determined as described in above sections.

Statistical analysis

Data were analyzed using linear models followed by analysis of variance (ANOVA). Continuous data such as plant biomass, herbivore growth, metabolites, volatile compounds, and gene expression levels were analyzed using linear models followed by ANOVA. For discrete data, such as gall numbers, virus copies, and bacterial counts, generalized linear mixed models (GLMMs) with a Poisson distribution followed by ANOVA were used. When necessary, pairwise or multiple comparisons of least squares means (LS means) were corrected using the FDR method. PCoA of Bray-Curtis distances was employed to compare bacterial profiles between control and linalool treatments, with significant differences determined by Monte Carlo tests with 999 permutations. Pearson correlation was used to assess the associations between the expression of BX and JA biosynthesis genes, as well as the correlations between HDMBOA-Glc concentration and bacterial growth. All statistical analyses were performed using R version 4.2.0, utilizing packages including "car", "emmeans", "vegan", "RVAideMemoire", "pheatmap", "dplyr", "phyloseq", "edgeR", "multcomp", "corrplot", "lme4", "reshape2", "magrittr", and "tidyr".

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SUPPLEMENTARY MATERIALS

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Figs. S1 to S18; Data S1 to S4; MDAR Reproducibility Checklist

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Linalool-triggered plant-soil feedback drives defense adaptation in dense maize plantings

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Editor's summary

Maize plants emit a volatile gas called linalool, which can influence the growth and development of neighboring plants. Guo *et al*. found that at high planting densities, high linalool concentrations triggered neighboring plants to release benzoxazinoids into the soil (see the Perspective by Schandry and Becker). These compounds cause the soil microbiota to change composition, with knock-on effects for plants that are subsequently grown there. The altered microbiome enhances defense responses to herbivores but reduces plant growth. These findings demonstrate the implications of high planting density on multiple facets of the plant growth environment. —Madeleine Seale