PLANT SCIENCE

Plant roots sense soil compaction through restricted ethylene diffusion

Bipin K. Pandey[1]*, Guoqiang Huang[2]*, Rahul Bhosale[1], Sjon Hartman[3,4], Craig J. Sturrock[1], Lottie Jose[1], Olivier C. Martin[5], Michal Karady[6], Laurentius A. C. J. Voesenek[3], Karin Ljung[7], Jonathan P. Lynch[8], Kathleen M. Brown[8], William R. Whalley[9], Sacha J. Mooney[1], Dabing Zhang[2,10]†, Malcolm J. Bennett[1]†

Soil compaction represents a major challenge for modern agriculture. Compaction is intuitively thought to reduce root growth by limiting the ability of roots to penetrate harder soils. We report that root growth in compacted soil is instead actively suppressed by the volatile hormone ethylene. We found that mutant *Arabidopsis* and rice roots that were insensitive to ethylene penetrated compacted soil more effectively than did wild-type roots. Our results indicate that soil compaction lowers gas diffusion through a reduction in air-filled pores, thereby causing ethylene to accumulate in root tissues and trigger hormone responses that restrict growth. We propose that ethylene acts as an early warning signal for roots to avoid compacted soils, which would be relevant to research into the breeding of crops resilient to soil compaction.

Soil compaction affects global crop cultivation by reducing root penetration in both the upper and deeper soil layers (*1*). Modern agricultural practices have exacerbated soil compaction, largely because of intensification of operations leading to the deployment of heavier machinery and tillage practices (*2*, *3*), severely degrading ~65 million hectares of land globally (*4*). Compaction increases soil bulk density and reduces soil porosity, limiting the availability and transport of water and nutrients (*4*, *5*). The decrease in soil pore space, especially in large air-filled pores (Fig. 1, A to D, figs. S1 and S2, and movies S1 and S2), also restricts diffusion of gases between roots and the rhizosphere (*6*). To deal with compacted soils and penetrate cracks, roots are reported to undergo adaptive growth responses, including increased radial expansion of root tips (*1*). However, the predominant response of roots is cessation of growth, for which the mechanistic basis remains unclear. Here, we report that entrapped ethylene functions as a key signal regulating root growth in compacted soils.

Ethylene is produced by root tissues, and its level increases when roots are exposed to compacted soil (*7*, *8*). Ethylene concentrations outside the root could increase as a result of the reduction in soil pore space in compacted soil, which affects gas diffusion from root tissues (Fig. 1, A to D, and figs. S1 and S2). To test this "restricted gas diffusion" model, we used the EIN3-GFP (green fluorescent protein) *Arabidopsis* ethylene response reporter (*9*) (fig. S3, A to C) and examined the effect of covering root tips with a gas-impermeable barrier. In agreement with model assumptions, restricting gas diffusion from root tip tissues triggered a rapid and sustained increase in EIN3-GFP in root elongation zone cell nuclei relative to controls (Fig. 1F versus Fig. 1E; fig. S3, D to G). This result is consistent with (i) limitation of ethylene release from root tip tissues and (ii) changes in gas diffusion rate between roots and the external environment, inducing ethylene accumulation and signaling. To rule out that changes in ethylene signaling were related to reduced oxygen levels in root tip tissues, we treated roots expressing the hypoxia markers *pPCO1:GFP-GUS*, *pPCO2:GFP-GUS* (*10*), and *RAP2.12-GFP* (*11*) with the gas-impermeable barrier. Hypoxia reporters were not induced by the gas barrier but were induced by submergence (figs. S4 to S6). We conclude that EIN3-GFP induction results from restricted ethylene diffusion rather than from hypoxic conditions (*11*).

Roots exposed to elevated levels of ethylene exhibited growth inhibition (Fig. 1, I and J), which phenocopied the impact of soil compaction (Fig. 1, G and H). We observed that rice roots grown in 1.1 g cm^{-3} (uncompacted) versus 1.6 g cm^{-3} (compacted) soil bulk densities exhibited reduced root length when exposed to compacted conditions (fig. S7, A and B). Root anatomical analysis revealed that compaction caused a factor of 3 decrease in epidermal cell length (fig. S7C), matched by a factor of 3 increase in cortical cell diameter (compare Fig. 1, G and H, and fig. S7D). Similarly, ethylene treatment reduced root length from mechanical impedance, but instead represents a timely response controlled by ethylene, perhaps to avoid growth in compacted soils (*14*). To discriminate between the effects mediated by mechanical impedance and by ethylene, we compared their impact on root tip shape. Soil compaction caused wild-type rice roots to double in width and their root caps to develop a "flattened" shape (compare Fig. 2, H and I). Soil compaction–induced radial growth and root cap shape changes were blocked in *osein2* (Fig. 2, J, K, and O). Hence, root tip shape changes induced by soil compaction appear to be controlled primarily by ethylene and not by mechanical impedance. Indeed, ethylene treatment alone was sufficient to trigger equivalent changes in root width (Fig. 1, I and J, and fig. S8, B and C) and cap shape (Fig. 2, L to N, and fig. S20) similar to roots exposed to soil compaction. Therefore, in plants, ethylene represents a critical signal controlling shape changes that underpin root compaction responses.

Given ethylene's functional importance during root responses to compaction, we investigated whether soil mechanical impedance triggered increased ethylene signaling in root tissues. We used transgenic *Arabidopsis* and rice expressing either an ethylene biosensor featuring *EIN3* (*9*) or *OsEIL1* sequences fused with GFP (fig. S21). In uncompacted soil, *35S:EIN3-GFP* or *proOsEIL1:OsEIL1-GFP* reporters in root nuclei were not detectable (Fig. 3, A and D). However, when reporter lines were grown in compacted soil, both ethylene reporters were detected in root elongation zone cells (Fig. 3, B, C, and E). To probe the role of ethylene in other soil types, we grew rice reporter lines in two contrasting soils. Compaction triggered a root ethylene response in clay soil (figs. S21 and S22) and sandy loam soil (Fig. 3E and fig. S23). Hence, the ethylene-based compaction mechanism appears to operate in different soil types.

How does soil compaction induce elevated ethylene signaling in root tissues? Mechanical impedance could cause roots to up-regulate ethylene synthesis. Profiling of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in excised rice root tips detected no change in levels after growth in compacted soil versus uncompacted controls (fig. S24). Alternatively, plant roots may sense soil compaction

Fig. 2. Disrupting ethylene response in rice confers root growth resistance to compacted soil. (**A** to **F**) CT images of primary roots of wild-type (WT) [(A) and (B)], *osein2* [(C) and (D)], and *oseil1* [(E) and (F)] in 1.1 BD [(A), (C), and (E)] versus 1.6 BD [(B), (D), and (F)]. (**G**) Violin plots of primary root length in uncompacted (1.1 BD) versus compacted (1.6 BD) conditions for WT, *osein2*, and *oseil1* rice seedlings. (**H** to **K**) Representative images showing root cap area in WT [(H) and (I)] and *osein2* [(J) and (K)] in 1.1 BD [(H) and (J)] versus 1.6 BD [(I) and (K)] soils. (**L** and **M**) Ethylene treatment of WT roots showing reduction in root cap area [(M) versus (L)]. (**N**) Violin plots showing reduction of root cap area after ethylene treatment. (**O**) Violin plots showing reduction of root cap area of WT but not *osein2* roots when grown in 1.6 BD versus 1.1 BD soils. Columella cells are marked in red [(L) and (M)]. $^*P \leq 0.05$, $^{**}P \leq 0.001$, $^{***}P \leq 0.0001$ (Student's *t* test). Scale bars,t

We directly tested whether soil compaction restricted gas diffusion by experimentally measuring ethylene's ability to move through compacted versus uncompacted soil. A 1-cm-thick soil column (connecting two air-filled chambers) was either left empty (control) or filled with uncompacted soil (1.1 g cm^{-3}) or compacted soil (1.6 g cm^{-3}) (Fig. 3I and fig. S25B). Ethylene was injected into the upper chamber (an increase in pressure was avoided) and ethylene concentrations were subsequently measured over time in the lower chamber until an equilibrium was reached between the chambers. In agreement with gas diffusion simulations, ethylene levels rapidly reached an equilibrium with the lower chamber in control conditions without soil resistance (Fig. 3I). Ethylene was also able to diffuse through uncompacted soil, albeit more slowly than the empty control by a factor of 10 to 50; in contrast, ethylene was unable to diffuse through compacted soil and was still undetectable in the lower chamber at 20 days (Fig. 3I). This result demonstrates that soil compaction (and the associated increase in soil moisture due to decreased porosity) affects ethylene diffusion rates, consistent with our restricted gas diffusion model. This much slower ethylene diffusion in compacted soil results in an enhanced ethylene response in root cells. This entrapped ethylene gas provides a fast and reliable signal for plants to interact with their environment, because nearly all roots produce ethylene under normoxic conditions (*15*).

Our results reveal how roots regulate growth responses to soil compaction. First, the inhibition of root growth by compacted soils is triggered by ethylene signaling, rather than simply by mechanical forces. Second, rather than using a dedicated mechano-perception mechanism, roots appear to sense soil compaction through restricted diffusion of this gaseous signal from the plant cells to the soil, causing ethylene to accumulate in root expansion zone cells and inhibiting elongation growth. Third, compaction and soil moisture status appear to have an impact on root elongation, not only because they control soil strength, but also through regulating ethylene diffusion. Fourth, we propose that ethylene acts as an early warning signal for roots to avoid compacted soils (*14*); if so, this could provide a pathway for how breeders might select crops resilient to soil compaction.

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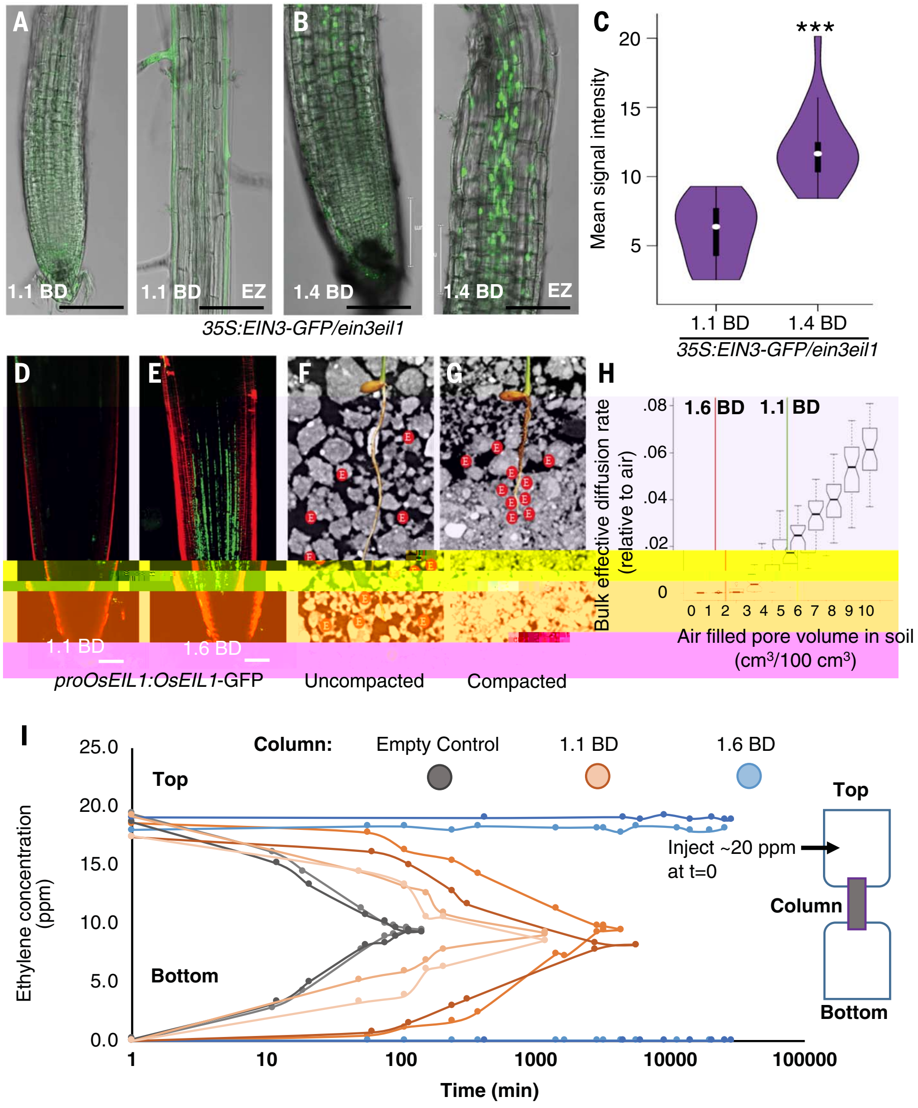

Fig. 3. Compacted soil reduces ethylene diffusion and enhances root ethylene signaling. (**A** and **B**) *Arabidopsis* ethylene reporter EIN3-GFP exhibits no nuclear GFP signal when grown in uncompacted soil (1.1 BD) (A) but is clearly detected in root EZ (elongation zone) cells when grown in compacted soil (1.4 BD) (B). (**C**) Violin plot of GFP signal in 1.1 BD versus 1.4 BD soil in EZ of *35S:EIN3-GFP/ein3eil1*. (**D** and **E**) Relative to 1.1 BD soil (D), a rice OsEIL1-GFP–based ethylene translational reporter exhibits elevated signal in compacted (1.6 BD) soil (E). (**F** and **G**) Schematic figures of ethylene diffusion (denoted by red circles) in uncompacted (F) versus compacted (G) soil, illustrating preferential accumulation of ethylene around and in root tissues. (**H**) Model simulation showing rate of bulk diffusion of ethylene in soil pores in uncompacted (green line) and compacted (red line) soil. (**I**) Graphical representation of quantification of ethylene across 1.1 BD and 1.6 BD soil layers (1 cm). After 20 ppm of ethylene was injected in the top chamber, we used gas chromatography–mass spectrometry to measure ethylene diffusion in the bottom chamber across empty, uncompacted (1.1 BD), and compacted (1.6 BD) soils. ***$P \leq 0.0001$ (Student's *t* test).

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SUPPLEMENTARY MATERIALS

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Materials and Methods
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References (*16*–*20*)
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Ethylene aplenty signals soil compaction

It's tough to drive a spade through compacted soil, and plant roots seem to have the same problem when growing in compacted ground. Pandey *et al.* found that the problem is not, however, one of physical resistance but rather inhibition of growth through a signaling pathway. The volatile plant hormone ethylene will diffuse through aerated soil, but compacted soil reduces such diffusion, increasing the concentration of ethylene near root tissues. The cellular signaling cascades triggered by too much ethylene stop root growth. Therefore, gaseous diffusion serves as a readout of soil compaction for plant roots growing in search of productive nutrition.

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