

Directional hydraulic stress indices predict root growth and hormonal reprogramming in plants

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Plants navigate fluctuating fluid environments in both soil and hydroponic systems, yet the physical principles by which roots transduce directional hydrodynamic forcing into developmental responses remain poorly quantified. By combining a flow-configurable microfluidic platform (P-ROOT) with fluid-structure interaction modeling and multiscale phenotyping, we establish a dimensionless, direction-dependent hydraulic stress index, defined as the ratio of flow-induced stress to root stiffness, that predicts growth-permissive and stress-adaptive hydraulic loading regimes in *Brassica juncea* roots. Our results reveal a pronounced directional asymmetry: Under parallel flow, root elongation is sustained across a broad hydraulic loading range, whereas counter-flow concentrates stress near the root apex, restricting the growth-permissive range and reducing elongation. Transitions across these hydraulic loading regimes coincide with coordinated cortical remodeling, auxin redistribution, reduced nitrogen uptake, and enhanced nitro-oxidative signatures near the root tip. These findings indicate that root developmental plasticity is governed more strongly by localized stress amplification than by the overall magnitude of hydraulic loading. By establishing hydraulic stress indices as predictive metrics linking mechanical loading to multiscale biological responses, this work provides a quantitative framework for understanding plant-fluid interactions and for designing flow-managed cultivation systems that optimize nutrient delivery.

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I. INTRODUCTION

Root development during germination is governed not only by biochemical signaling networks [1] but also by mechanical forces arising from the physical microenvironment [2–10]. Hormones such as auxin and abscisic acid regulate cell elongation, morphology, and stress adaptation [11–14], while root-soil mechanical interactions further shape developmental trajectories [15,16].

In soils and hydroponic systems, fluid motion generated by infiltration, drainage, or nutrient delivery imposes hydraulic stresses on the root surface [17,18]. These stresses—collectively termed hydraulic stress or flow-induced stress (FIS)—influence cell expansion, wall mechanics, and mechanosensitive signaling pathways [19,20]. Here, “hydraulic stress” refers to the external mechanical load exerted by flow, whereas “FIS” denotes its modeled or experimentally quantified magnitude. Despite increasing recognition of plant mechanosensitivity, a quantitative framework linking *direction-dependent* hydraulic forcing to developmental responses and hormonal reprogramming remains lacking.

A central unresolved question is whether roots respond primarily to the magnitude of flow-induced stress or to its spatial localization imposed by flow direction. In natural and engineered environments, directional hydraulic loading produces asymmetric deformation: The flow-facing side experiences

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elevated mechanical stress, whereas the leeward side experiences reduced loading. Such asymmetry can reorganize cell walls, redirect microtubules, and bias auxin fluxes, thereby influencing growth orientation and stress adaptation. Yet the hydraulic loading ranges associated with growth-permissive and stress-adaptive responses remain poorly defined.

Existing soil and hydroponic systems lack the spatiotemporal precision required to decouple mechanical from chemical or microbial cues [21–27]. Although microfluidic platforms have enabled controlled studies of root-environment interactions [28–32], most impose spatially uniform flows and do not quantify direction-dependent mechanical stresses. Earlier systems such as the RootChip [22] and dual-flow RootChip [33] enabled chemical asymmetry but lacked quantitative mapping of hydraulic loading. Consequently, the combined effects of flow direction and rate on root morphogenesis remain unresolved, and the hydraulic stress indices associated with developmental responses are unknown.

To address these gaps, we developed the Phytofluidics-based Root Orientation and Optimization Tool (P-ROOT), a flow-configurable microenvironment that enables controlled parallel (PF) and counterflow (CF) configurations while allowing quantitative stress mapping and live imaging. Earlier on-chip studies from our group demonstrated that nutrient flow modulates root physiology [34], but the mechanical basis and quantitative limits of adaptation were not resolved. Here, by integrating directional flow manipulation with fluid-structure interaction (FSI) modeling, we establish a dimensionless hydraulic stress index, defined as a measure of flow-induced stress relative to root stiffness, that provides a quantitative framework for relating hydraulic loading to root developmental responses.

Using live imaging, histology, hormone quantification, nutrient uptake assays, Raman spectroscopy, and finite-element modeling, we demonstrate that roots exhibit distinct hydraulic stress index ranges under parallel and counterflow conditions that are associated with differences in elongation, structural remodeling, and biochemical reprogramming. Crossing these hydraulic stress index ranges coincides with cortical compaction, auxin overaccumulation, nitro-oxidative signaling, and reduced nitrogen uptake, revealing coordinated multiscale responses to directional hydraulic loading.

By establishing quantitative hydraulic stress indices that link physical forcing with hormonal and metabolic responses, this work advances a quantitative framework for plant mechanobiology and provides design principles for flow-controlled cultivation systems that sustain growth while minimizing stress-induced trade-offs.

II. MATERIALS AND METHODS

A. Plant material and seed germination

Seeds of *Brassica juncea* (cv. Pusa Jaikisan; ICAR-IARI, Karnal, India) were surface sterilized in 70% (v/v) ethanol for 30 s, followed by 4% (w/v) sodium hypochlorite for 5 min, and rinsed five times with autoclaved double-distilled water. Sterilized seeds were placed on Grade-1 Whatman filter paper moistened with sterile distilled water, sealed with Parafilm, and incubated in darkness at 26 °C and 75% rel-

ative humidity for 48 h. Germinated seedlings were then grown under a 16/8 h light/dark cycle (~3000 lux) at identical temperature and humidity until transferred to P-ROOT [35]. Full sterilization and germination protocols are detailed in the Supplemental Material, Materials and Methods, Sec. II A, including chamber setup and illumination parameters [36].

B. P-ROOT microdevice fabrication and flow regulation

The P-ROOT device was fabricated using polydimethylsiloxane (PDMS) (SYLGARD™ 184, Sigma Aldrich) with a wire-drawing technique based on a 3D-printed ABS master. Each channel measured 0.8 mm × 0.8 mm in cross section and 30 mm in length, consisting of a 12 mm chamber to accommodate early root elongation. Two-day-old seedlings were aseptically transferred to the P-ROOT. Full-strength Murashige-Skoog medium was perfused via Harvard Apparatus PHD ULTRA™ Syringe Pump to impose PF and CF regimes. A *no-flow* (NF) condition (stagnant) was maintained as an experimental control. Flow remained laminar up to 1.2 ml h⁻¹ (Re < 1). See schematic shown in Figs. 1(a)–1(d). Additional fabrication parameters, curing steps, and assembly diagrams are provided in the Supplemental Material, Sec. II B, Fig. S1 [36].

C. Flow regime characterization and live imaging

Experiments were conducted for 12 h after transfer of seedlings for all characteristic flow rates: $Q_{\min} = 0.05$ ml h⁻¹ to $Q_{\max} = 1.20$ ml h⁻¹. Imaging was performed using a Leica DMI3000 M inverted microscope equipped with a Phantom Miro LAB320 high-speed camera (1900 × 1200 pixels; 2.5× to 10× objectives). Bright-field images were acquired every 2 h for growth analysis (Figs. 1 and 2). Complete set of images of roots, imaging parameters, and acquisition settings are listed in the Supplemental Material, Secs. II C and II D, Fig. S2 [36].

D. Image processing and quantitative growth analysis

Root lengths were quantified from micrographs using ImageJ/Fiji, and root-length—flow-rate curves and heatmaps were generated in MATLAB R2022a. The experimental root-length data were represented using the Piecewise Cubic Hermite Interpolating Polynomial (PCHIP) method. Confidence intervals were estimated using Student's *t* distribution at the 90% confidence level based on biological replicates (*n* = 3). The flow-rate range and corresponding τ_{ROI}/E range were defined as the intervals over which the PCHIP-interpolated root-length response remained above the lower 90% confidence bound of the maximum observed mean root length.

Violin plots and distribution fittings were visualized using Python (NumPy, Matplotlib, Seaborn). The region of interest (ROI) and replicate counts were predefined, and analyses were performed in a blinded manner. Statistical comparisons used one-way Analysis of Variance (ANOVA) followed by Duncan's multiple-range test (*p* < 0.05). Processing workflows are described in the Supplemental Material, Secs. II D and III A–III C [36].

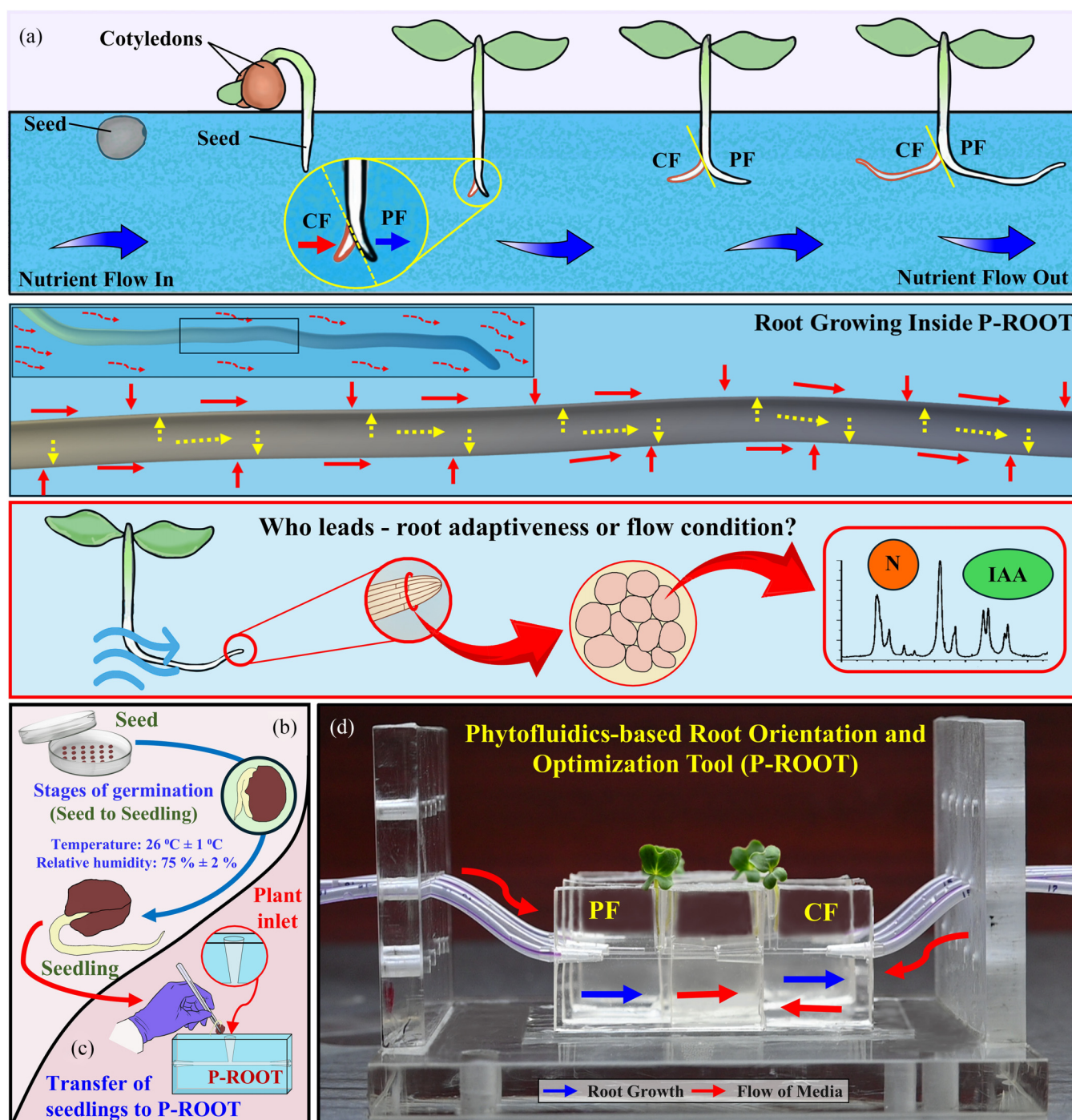


FIG. 1. Directional flow regimes establish a quantitative framework for root stress analysis. (a) Conceptual schematic illustrating seed germination, early root emergence, and exposure to parallel (PF, blue) and counter (CF, red) directional flow fields, highlighting mechanical stress asymmetries along the root axis. The middle panel shows nutrient advection and localized mechanical loading along the root surface. The bottom panel poses the central question of whether root adaptiveness or imposed flow dominates downstream biochemical responses, where N denotes nitrogen and IAA denotes the auxin indole-3-acetic acid. (b) Workflow for seed germination and transfer to the P-ROOT device. (c) Loading of seedlings into PDMS-based microchannels to maintain sterile, stable flow. (d) Photograph of the P-ROOT platform with PF and CF configurations under laminar flow ($Re < 1$), demonstrating how P-ROOT couples controlled directional hydraulics with quantitative mechanical mapping for subsequent FIS and internal flow-induced stress (iFIS) analysis.

E. Histological imaging and morphometric analysis

Roots were harvested after 12 h, embedded in agar, hand sectioned, and stained with safranin. Sections were

dehydrated and imaged by bright-field microscopy. Cross-sectional area, stelar region area, cortical cell counts, and single-cell area distributions were quantified using ImageJ

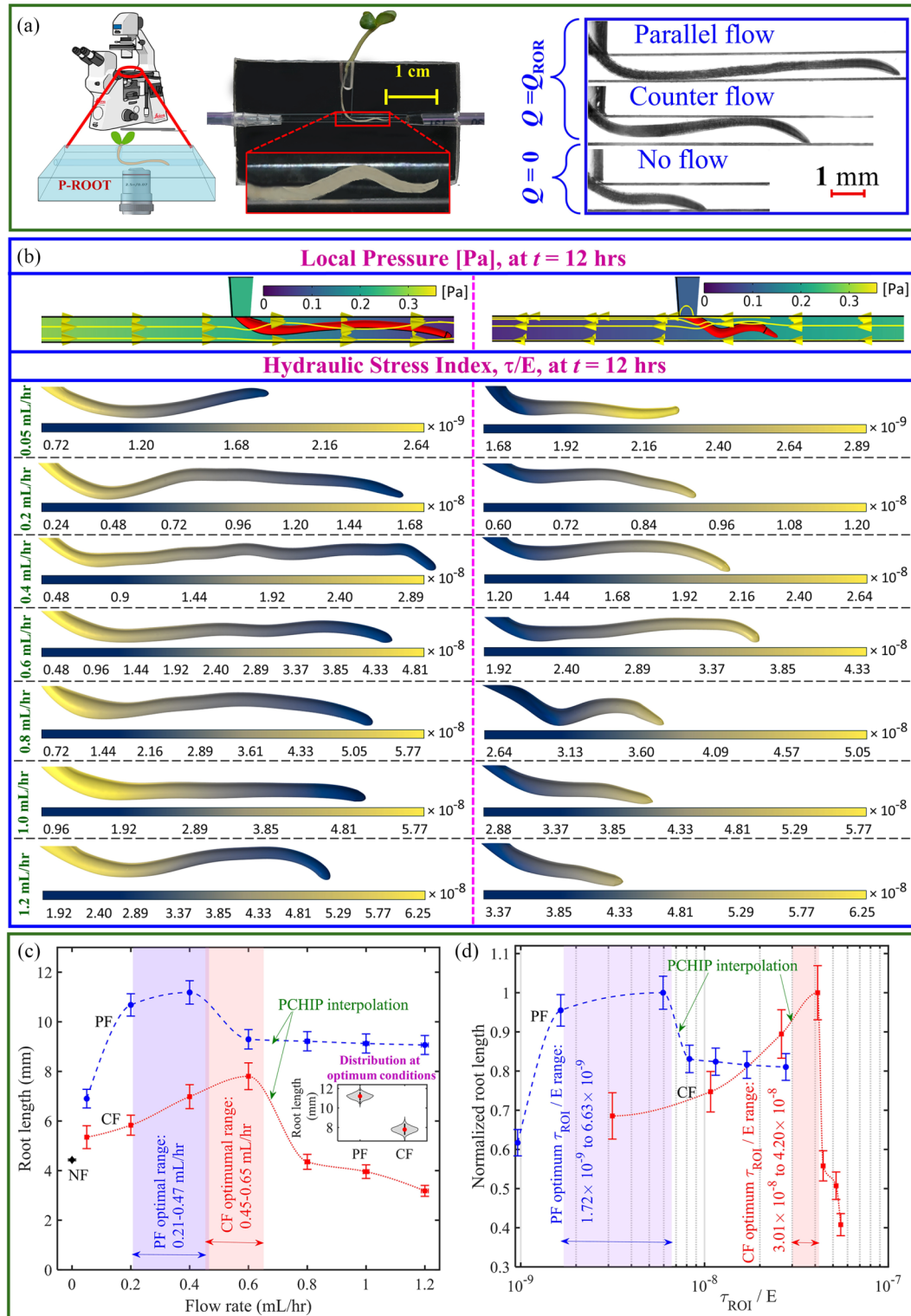


FIG. 2. Direction-dependent hydraulic stress indices are associated with root growth responses. (a) Representative root morphologies after 12 h under PF, CF, and NF, showing enhanced elongation under PF and reduced growth under CF ($n = 3$ biological replicates). (b) Fluid-structure interaction contours of pressure and hydraulic stress index (τ/E), showing distributed loading under PF and apex-localized stress amplification under CF. (c) Root length as a function of flow rate for PF, CF, and NF conditions. Curves were obtained using piecewise cubic Hermite interpolation; shaded regions indicate 90% confidence-based optimal flow-rate intervals, and inset violin plots show the corresponding root-length distributions. (d) Normalized root length vs the ROI-averaged hydraulic stress index (τ_{ROI}/E). Shaded regions indicate confidence-based optimal stress-index ranges associated with maximal root growth.

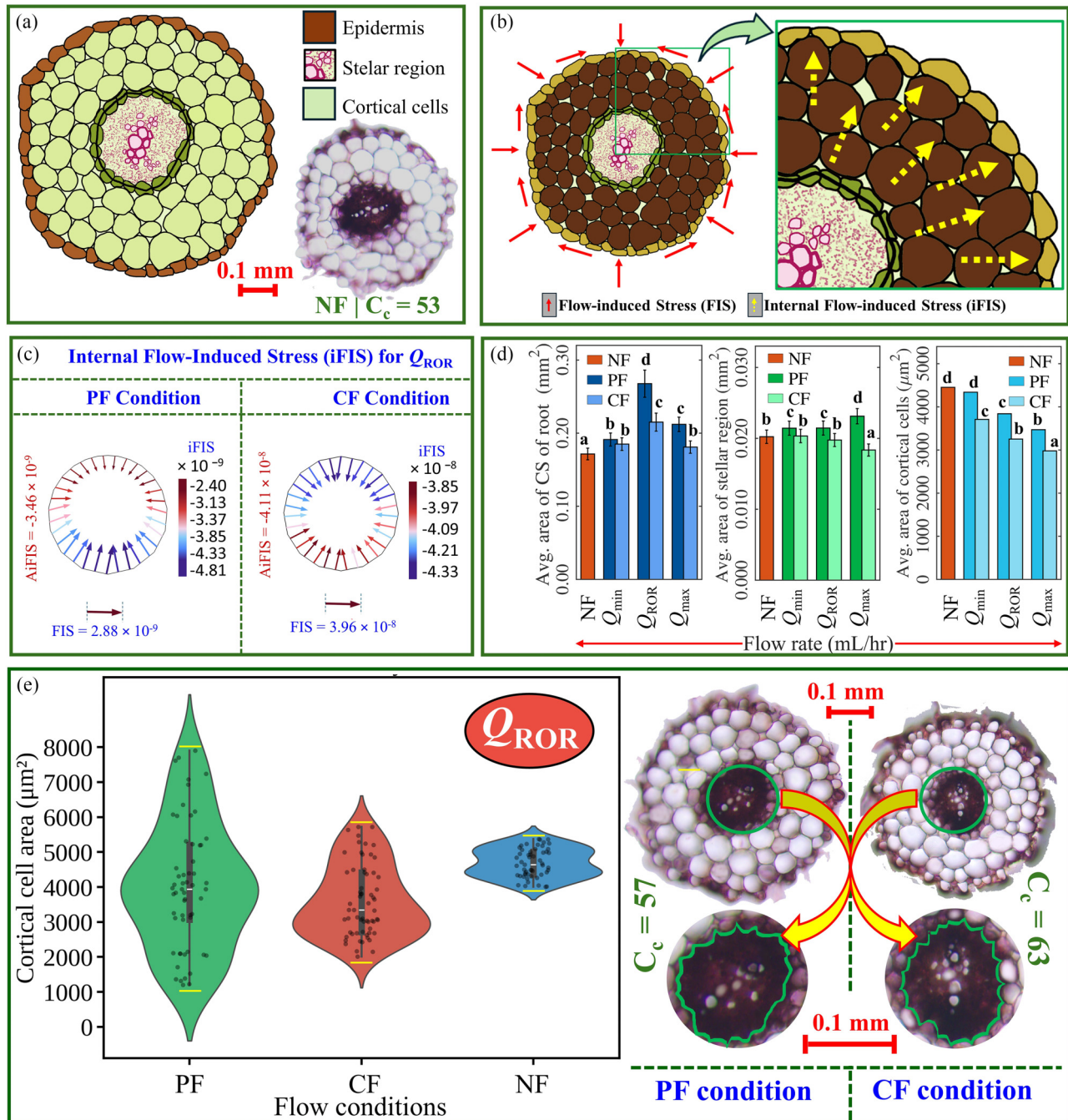


FIG. 3. Coordinated root adaptations under direction-dependent hydraulic loading. (a) Schematic of the root cross section with a representative NF image defining analyzed regions at the ROI. (b) Conceptual diagram illustrating how external FIS propagates into internal stress (iFIS) across tissue layers during fluidic stimulation. (c) Finite-element maps of iFIS in the ROI at Q_{ROR} under PF and CF, illustrating distributed internal loading under PF and localized stress amplification near the stele under CF. (d) Morphometric analysis of total root cross-sectional area, stele area, and mean cortical cell area under NF, PF, and CF conditions across the investigated flow rates ($p < 0.05$, $n = 3$). (e) Violin plots of cortical cell-area distributions at Q_{ROR} . PF maintains relatively symmetric cell-size distributions, whereas CF exhibits a distribution shifted toward smaller cortical cell areas. NF roots display a narrow, centered distribution characteristic of unstressed conditions. Complete anatomical datasets and statistical analyses are provided in the Supplemental Material, Figs. S3 and S5, and Table S1 [36].

[Figs. 3(a)–3(e)]. For each root, cortical cell measurements were summarized at the root level before statistical comparison. The independent biological replicate was the individual root ($n = 3$ roots per condition); individual cells measured within a root were treated as nested

technical observations and were not considered independent biological replicates. Extended histology, Lorentz fitting results, and statistical metrics appear in the Supplemental Material, Secs. II E and III E, Figs. S3 and S5, and Table S1 [36].

F. Auxin quantification

Fresh tissue (~ 100 mg) was extracted with methanol and analyzed by liquid chromatography-mass spectrometry (LC-MS) in negative electrospray ionization mode using external IAA calibration. IAA content was normalized to tissue fresh weight (FW). Auxin concentration was expressed as pmol mg^{-1} fresh weight. Extraction solvents, gradient conditions, and calibration standards are described in the Supplemental Material, Sec. II F [36].

G. Nitrogen uptake assay (TKN method)

Shoot and root tissues were oven dried and analyzed using the Total Kjeldahl Nitrogen (TKN) method (KES 08L R analyzer). Nitrogen content across the flow regimes is shown in Fig. 4(c). Digestion, calibration, and normalization procedures are provided in the Supplemental Material, Sec. II G [36].

H. Raman spectroscopy

Raman spectra were collected from the tip-meristem region ($n = 3$, 2–4 positions per root) using a Horiba LabRAM HR spectrometer (514.5 nm laser, 10 s acquisition). Stress-linked bands at $1512\text{--}1540\text{ cm}^{-1}$ corresponding to nitro-oxidative species were quantified and analyzed by principal component analysis (PCA) [Fig. 4(b)]. Extended setup appears in the Supplemental Material, Sec. II H and Fig. S4, with full peak assignments in Table S2 [36].

I. Statistical analysis

All values are reported as mean $\pm$ standard deviation, unless stated otherwise. Exact sample sizes (n) are provided in the main text and represent independent biological replicates. For histological measurements, the individual root was treated as the experimental unit. Multiple cortical cells measured within the same root were considered nested observations and were summarized at the root level before statistical analysis. Cell-level measurements were used only to visualize the distributions. One-way ANOVA followed by Duncan's *post hoc* test was performed using IBM SPSS Statistics v29, with statistical significance defined as $p < 0.05$. Exact sample sizes, statistical groupings, effect sizes, Lorentzian fitting procedures, and normality assessments are provided in the Supplemental Material, Sec. III, Table S1, and Fig. S5 [36]. Figures were prepared using OriginPro 2024 and MATLAB R2022a.

J. Fluid-structure interaction simulations

Flow fields and mechanical stresses were simulated using COMSOL Multiphysics[®]. The incompressible, steady-state Navier-Stokes equations were coupled to a linear elastic solid representing the root, with no-slip boundary conditions imposed at the channel walls and root surfaces, a uniform inlet velocity corresponding to each experimental flow rate, and zero gauge pressure at the outlet. The elastic modulus was set to $E = 4.1625$ MPa and Poisson's ratio to $\nu = 0.49$, consistent with the hydrated cortical tissue stiffness of *B. juncea* measured by atomic force microscopy (AFM). Varying E

within reported literature ranges did not change the order of magnitude of the resulting τ/E hydraulic stress indices. Simulation equations, solver details, and mesh statistics are provided in the Supplemental Material, Sec. IV [36]. Derived surface FIS and iFIS maps are shown in the Supplemental Material, Figs. S6–S8 [36].

The local value of τ/E varies spatially throughout the root and therefore captures heterogeneity in the FSI-derived stress field. The ROI-averaged quantity τ_{ROI}/E reported in Fig. 2 represents an area average over the ROI near the root tip, not an average over the entire root. Because this ROI contains the biologically relevant meristematic region, τ_{ROI}/E was used to characterize the biomechanical loading environment experienced by this region. Local stress amplification remains represented in the underlying spatial τ/E field. Definitions of the local hydraulic stress index (τ/E) and the ROI-averaged hydraulic stress index (τ_{ROI}/E) are provided in the Supplemental Material, Sec. IV [36].

III. RESULTS

Understanding how roots integrate directional hydraulic cues with growth and stress adaptation requires a framework that bridges physics and physiology. To address this challenge, we combined live imaging, histology, hormone and nutrient assays, Raman spectroscopy, and finite-element fluid-structure interaction modeling within the P-ROOT platform. This cross-scale integration enabled us to connect external flow conditions to internal mechanical loading, to identify hydraulic loading ranges associated with distinct growth responses, and to trace the resulting structural and biochemical reprogramming. Our analyses reveal a hierarchy of flow-dependent responses—from elongation to reinforcement to signaling modulation—associated with direction-dependent hydraulic loading environments.

A. Directional hydraulic stress indices and growth responses

To resolve how hydraulic forcing regulates root development, we employed the P-ROOT microfluidic platform to impose controlled parallel flow, counterflow, and no-flow conditions [Figs. 1(a)–1(d); Supplemental Material, Fig. S1 [36]]. Laminar flow regimes ($\text{Re} < 1$) were confirmed numerically (Supplemental Material, Fig. S1 [36]), consistent with classical low-Reynolds-number microfluidic transport theory. Earlier platforms such as the RootChip [22] and existing microfluidic platforms [28–30] rarely impose asymmetric flows that mimic natural hydrodynamic heterogeneity. Here, we integrate fluid-structure interaction modeling to explicitly map flow-induced stress and its internal transmission under directional hydraulic forcing. As schematized in Fig. 1(a), PF aligns fluid motion with root elongation, whereas CF opposes elongation and generates a stagnation region at the apex. These two configurations therefore impose qualitatively different mechanical *stress distributions*: PF distributes shear and pressure gradients longitudinally along the root surface, while CF concentrates normal and shear loading near the tip. Such directional stress asymmetries are expected to bias mechanosensitive growth responses and hormone transport,

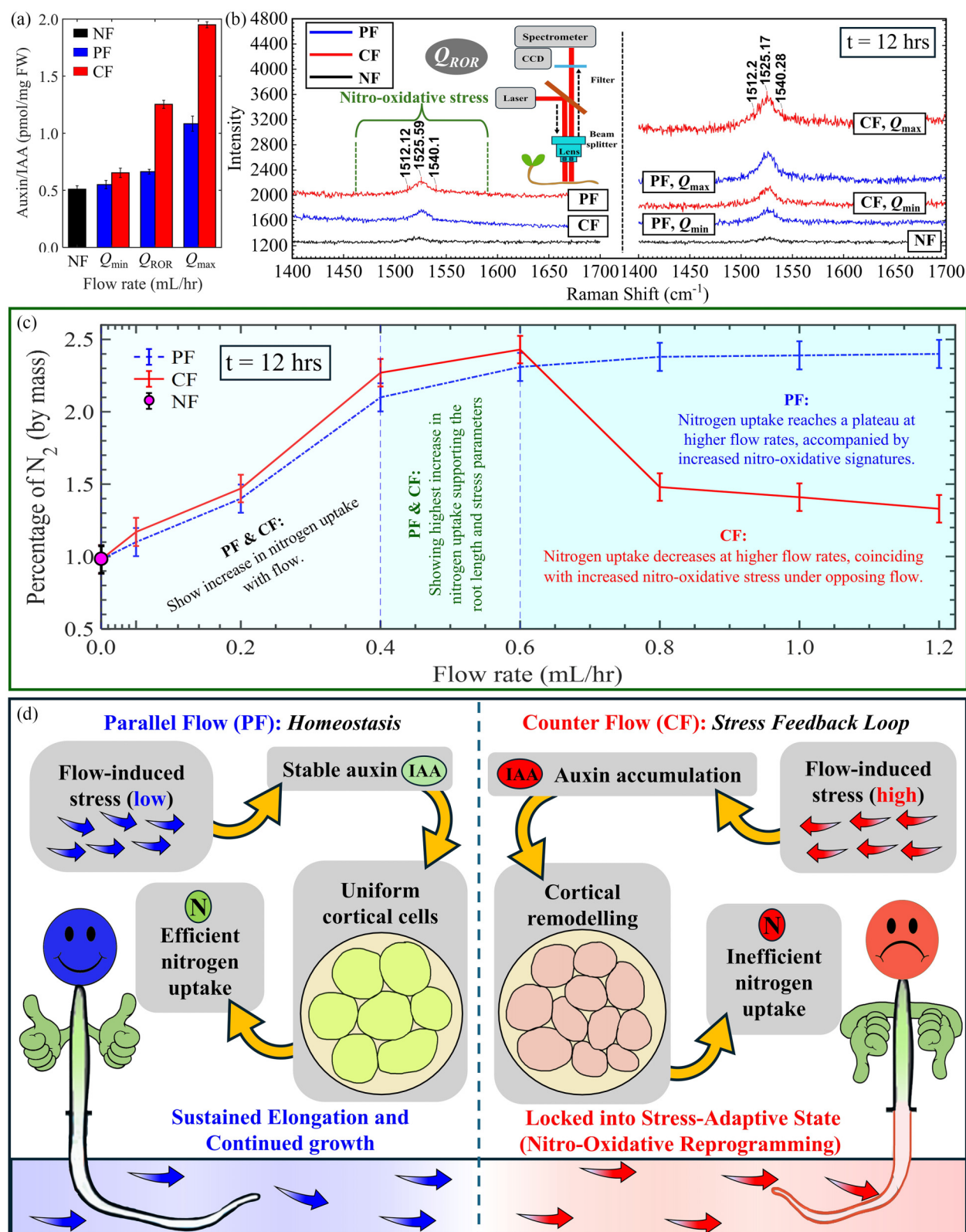


FIG. 4. Biochemical and physiological responses to direction-dependent hydraulic loading. (a) IAA concentrations after 12 h under NF, PF, and CF conditions ($n = 8$). IAA remains relatively stable under PF but increases markedly under CF at $Q_{\max}$. (b) Root-tip Raman spectra under NF, PF, and CF. The highlighted 1512–1540 cm^{-1} region contains stress-associated bands tentatively assigned to nitroaromatic and nitrosothiol species; the strongest signal occurs under CF at $Q_{\max}$. (c) Nitrogen content after 12 h across the investigated flow rates ($n = 16$). Values increase and plateau under PF but decrease beyond Q_{ROR} under CF. (d) Conceptual summary linking distributed loading under PF and localized stress amplification under CF to structural, hormonal, spectroscopic, and nitrogen responses. Additional Raman details are provided in the Supplemental Material, Fig. S4 and Table S2 [36].

consistent with broader concepts of mechanically induced developmental regulation [37–41].

A central question in plant mechanobiology is whether roots respond primarily to the magnitude of hydraulic forces or to their *direction*. Mechanical stress is known to modulate elongation and tissue remodeling [34,40–42], yet previous systems could not independently control flow rate and flow orientation. As a result, it has remained unclear whether growth inhibition reflects total hydraulic loading or the asymmetric stresses imposed by directional flow. Using the P-ROOT platform, we decouple these variables and quantify hydraulic stress indices associated with distinct root-growth responses under directional flow conditions.

Representative root morphologies after 12 h [Fig. 2(a); Supplemental Material, Fig. S2 [36]] reveal strong direction dependence: parallel flow consistently produces longer roots than counterflow, while no flow supports only modest elongation. Fluid-structure interaction simulations provide the mechanical context [Fig. 2(b); Supplemental Material, Figs. S6–S8 [36]]. Under PF, the root experiences a smooth, longitudinally distributed pressure gradient and shear field along its surface, resulting in relatively uniform external loading across the elongation zone. In contrast, CF concentrates both pressure and shear at the root apex, generating localized stress hotspots that sharply elevate mechanical load at tip tissues. Internal FIS (iFIS) distributions further confirm that PF produces broadly homogeneous internal stresses, whereas CF drives pronounced tip-focused stress amplification (Supplemental Material, Figs. S6–S8 [36]). Thus, directional flow reshapes the spatial distribution of hydraulic loading, establishing distinct mechanical environments along the root axis.

To quantify these mechanical environments, we define a local hydraulic stress index τ/E as a dimensionless measure of flow-induced stress relative to root stiffness:

$$\frac{\tau}{E} = \frac{\sqrt{\tau_n^2 + \tau_t^2}}{E}, \quad (1)$$

where τ_n and τ_t are the local normal and tangential internal stress components inside the root, respectively, and E is the equivalent modulus of elasticity measured by atomic force microscopy. We note that τ/E should not be interpreted as the actual strain experienced by the tissue, which additionally depends on geometry, boundary conditions, and constitutive behavior. Rather, τ/E provides a dimensionless hydraulic stress index that quantifies the relative magnitude of flow-induced mechanical loading with respect to root stiffness.

Because τ/E varies spatially throughout the root, we further define an ROI-averaged hydraulic stress index, τ_{ROI}/E , to characterize the biomechanical environment surrounding the region of interest:

$$\frac{\tau_{\text{ROI}}}{E} = \frac{1}{E} \frac{\int \tau dA_{\text{ROI}}}{\int dA_{\text{ROI}}}, \quad (2)$$

where A_{ROI} is the area of the defined root-tip region and dA_{ROI} is an elemental area within that region. Although local maxima of τ/E provide information regarding stress amplification, they are sensitive to spatial localization and numerical discretization. Therefore, τ_{ROI}/E provides a more robust metric for comparing PF and CF loading conditions while preserving directional differences in stress distribution.

These distinct spatial stress patterns generate different profiles of τ_{ROI}/E [Fig. 2(d)], enabling quantitative comparison of the hydraulic loading environments experienced by roots under directional flow.

To assess the robustness of this metric, the uncertainty associated with τ_{ROI}/E was estimated using first-order uncertainty propagation based on a Taylor-series expansion for independent variables [43,44], incorporating contributions from experimental measurements and numerical simulations. The resulting uncertainty estimates for all investigated conditions are provided in the Supplemental Material, Sec. III D [36].

Figure 2(c) shows absolute root length as a function of flow rate for PF, CF, and NF conditions, with $n = 3$ independent roots per condition. The root-length response was analyzed using the PCHIP interpolation method [45–47], which preserves the shape of the experimental data and is well suited for biological datasets with nonlinear trends. The optimal flow-rate range was identified from the interpolated response and the lower 90% confidence limit of the maximum observed root length using Student's t distribution [48–50], providing a statistically supported range rather than a single optimum value. Detailed mathematical information is provided in the Supplemental Material, Secs. III A and III B [36].

Both PF and CF exhibit hydraulic loading ranges associated with maximum root elongation, followed by a progressive decline in growth as hydraulic loading increases beyond these ranges. However, PF sustains elongation across a broader flow-rate interval, whereas CF exhibits a narrower hydraulic loading range associated with enhanced growth and a more pronounced reduction in elongation at higher flow rates. Using PCHIP interpolation and a 90% confidence criterion, the optimal flow-rate ranges were identified as 0.21–0.47 ml h^{−1} for PF and 0.45–0.65 ml h^{−1} for CF.

Because the confidence-based optimization analysis identified ranges rather than unique optimum values, a representative flow rate within each optimal range, denoted as Q_{ROR} (Representative Optimal-Range Flow Rate), was selected for subsequent histological, biochemical, and spectroscopic analyses. Based on the experimentally identified optimal ranges, Q_{ROR} was chosen as 0.4 ml h^{−1} for PF and 0.6 ml h^{−1} for CF. Representative root micrographs at Q_{ROR} under PF and CF conditions are shown in Fig. 2(a).

As shown in the inset of Fig. 2(c), root lengths within the PF and CF optimal flow-rate ranges differ significantly, with PF producing greater elongation than CF and NF. Statistical analysis (one-way ANOVA followed by Duncan's test; Supplemental Material, Table S3 [36]) further confirms significant differences between PF and CF root-growth responses across the investigated flow conditions, consistent with mechanically induced growth modulation reported in thigmomorphogenetic systems [41,42].

Crucially, plant mechanosensing is known to operate at the local, cellular scale, where steep strain gradients and membrane tension can arise even when average tissue strain is small. For example, transient mechanical perturbations of *Arabidopsis* roots produce Ca²⁺ signals that remain confined to the mechanically strained region and track the applied strain dynamics [51]. At the mechanistic level, mechanosensitive ion channels respond directly to membrane tension and may

preferentially activate at geometry-driven “hot spots” of stress concentration [52]. Thus, the separation between the PF and CF optimal hydraulic loading ranges is consistent with distinct stress environments (distributed loading versus tip-localized gradients), rather than a requirement for large-scale tissue deformation.

B. Coordinated root adaptations associated with elevated hydraulic loading

Having established direction-specific hydraulic stress indices associated with distinct root-growth responses, we next examined how root tissues reorganize under hydraulic loading conditions beyond the experimentally identified optimal ranges. Cortical thickening and anatomical reinforcement under soil compaction or hydraulic stress have been reported previously [53–55], yet whether such structural remodeling aligns quantitatively with mechanically defined stress indices remains unresolved.

Figure 3(a) defines the region of interest used for all cross-sectional analyses. Histological analysis was performed on $n = 3$ independent roots per condition. Full histological datasets are provided in the Supplemental Material, Fig. S3 [36]. Under NF conditions, roots exhibit relatively large, uniformly expanded cortical cells, consistent with diffusion-limited yet mechanically unstressed growth. Under PF at Q_{ROR} , cortical organization remains balanced, with moderate cell enlargement and preserved symmetry across tissues. In contrast, CF—particularly at elevated flow rates—induces pronounced architectural reorganization characterized by smaller, denser cortical cells and reduced cross-sectional areas.

The conceptual schematic in Fig. 3(b) illustrates how external flow-induced stress propagates inward as internal stress (iFIS) across epidermal, cortical, and stelar layers. Finite-element iFIS maps at 12 h [Fig. 3(c); Supplemental Material, Figs. S6–S8 [36]] reveal strong direction dependence: PF produces gradually decreasing internal stress along the elongation zone, whereas CF generates localized stress hotspots near the apex and stele. These elevated internal stresses spatially coincide with the cortical compaction observed under CF, linking stress localization to tissue-level remodeling. Such localization is consistent with models of plant mechanosensing in which spatially confined strain gradients initiate downstream signaling cascades and tissue remodeling, even when global deformation remains small [51,52].

Morphometric quantification confirms that structural parameters vary systematically with flow direction and hydraulic stress index [Fig. 3(d)]. PF yields the largest total and stelar cross-sectional areas and the greatest mean cortical cell size, consistent with sustained elongation under distributed hydraulic index. NF roots display moderate values with uniformly expanded cells, reflecting unstressed but diffusion-limited growth. In contrast, CF produces a marked decline across all metrics: total cross-sectional area and stelar region area decrease, and cortical cells become smaller and more densely packed, indicating localized reinforcement under concentrated tip loading.

Violin plots of cortical cell-size distributions at Q_{ROR} [Fig. 3(e)] further illustrate these distinctions. PF exhibits

relatively narrow, symmetric distributions, whereas CF displays broader, skewed distributions with significantly reduced median cell area. The cell-level distributions and Lorentzian fits shown in the Supplemental Material, Fig. S5 [36] provide a descriptive representation of the differences in cortical cell-size distributions among conditions. Statistical comparisons were based on root-level summary values from three independent roots per condition. Quantitatively, the average cortical cell area declines from $\sim 4450 \mu\text{m}^2$ in NF roots to $\sim 3840 \mu\text{m}^2$ in PF at Q_{ROR} , $\sim 3250 \mu\text{m}^2$ in CF at Q_{ROR} , and as low as $\sim 2980 \mu\text{m}^2$ in CF at Q_{max} .

These structural shifts are consistent with a trade-off between mechanical reinforcement and transport capacity. Cortical compaction under CF increases tissue density and likely enhances resistance to localized stress amplification, but reduces intercellular space and potentially constrains diffusional and hydraulic transport. Notably, although cross-sectional area at the defined ROI is smallest under NF and increases under flow conditions, whole-root morphology exhibits a different trend. In the absence of flow, diffusion-limited transport promotes broader radial expansion along the full root axis, yielding a larger effective diameter when the entire root is considered. This distinction highlights the difference between localized mechanical loading at the ROI and whole-root compensatory thickening under NF conditions.

The integration of histological metrics (Fig. 3; Supplemental Material, Fig. S3 [36]), statistical distribution analyses (Supplemental Material, Fig. S5; Table S1 [36]), and mechanical modeling (Fig. 2; Supplemental Material, Figs. S6–S8 [36]) demonstrates that hydraulic loading conditions outside the experimentally identified optimal ranges are associated not only with reduced elongation but also with coordinated structural reorganization. These anatomical responses are consistent with enhanced mechanical reinforcement under direction-dependent hydraulic loading and provide the structural context for the biochemical responses examined in the following subsection.

C. Biochemical reprogramming and feedback under stress

The hydraulic stress indices identified in the previous sections are associated with distinct growth responses and structural adaptations under directional hydraulic loading. Whether canonical biochemical stress pathways align with these mechanically defined hydraulic loading ranges has remained unclear.

Although roots are known to accumulate reactive oxygen and nitrogen species under abiotic stress [56–58], these biochemical signatures have not previously been quantified in relation to direction-dependent hydraulic stress indices. To determine whether biochemical reprogramming aligns with the local hydraulic stress indices resolved by P-ROOT [Fig. 2(b)], we integrated auxin quantification, Raman spectroscopy, and nitrogen uptake assays.

Auxin levels [Fig. 4(a)] exhibit trends that closely correspond to the hydraulic loading ranges identified from τ_{ROI}/E [Fig. 2(d)]. When the hydraulic stress index remains within the PF-associated τ_{ROI}/E range [1.72×10^{-9} to 6.63×10^{-9} ; Fig. 2(d)], auxin levels increase only modestly from ~ 0.66 to $\sim 1.1 \text{ pmol mg}^{-1} \text{ FW}$ between Q_{ROR} and Q_{max} ($n = 8$),

without coincident growth inhibition (Fig. 2; Supplemental Material, Fig. S2 [36]).

In contrast, once CF exceeds its optimal τ_{ROI}/E range [3.01×10^{-8} to 4.20×10^{-8} , Fig. 2(d)], auxin concentrations increase sharply, reaching $\sim 1.95 \text{ pmol mg}^{-1} \text{ FW}$ at $Q_{\max}$ [Fig. 4(a)]. NF roots maintain baseline auxin levels ($\sim 0.55 \text{ pmol mg}^{-1} \text{ FW}$), reflecting diffusion-limited yet mechanically unstressed growth.

The nearly threefold elevation in auxin under CF relative to PF and NF is consistent with a mechanically influenced redistribution of auxin under localized apex loading.

Given that mechanosensitive calcium signaling can be spatially restricted to mechanically perturbed regions [51], localized hydraulic stress provides a plausible mechanical correlate of the hormone redistribution observed here. Given that auxin transport and signaling are known to respond to mechanical cues [2,9] via cytoskeletal reorganization and PIN-FORMED (PIN)-mediated flux modulation [7,59–61], these results support a model in which spatially concentrated hydraulic stress biases hormone distribution [2] under conditions of elevated and spatially concentrated hydraulic loading.

Secondary metabolites play central roles in plant-environment interactions and stress adaptation [62]. Signaling molecules such as abscisic acid, salicylic acid, and nitric oxide (NO) mediate many of these responses [63]. Under mechanical or abiotic stress, perturbations in NO metabolism generate reactive nitrogen species, including nitrotyrosine ($\text{NO}_2\text{-Tyr}$), nitrosogluthathione, and nitrosothiols, which drive protein nitration and constitute hallmarks of nitro-oxidative stress [64–66].

Nitro-containing functional groups exhibit characteristic Raman-active vibrational modes arising from changes in bond polarizability, particularly symmetric and asymmetric NO_2 stretching in nitroaromatic structures and S–NO stretching in nitrosothiols. These modes typically fall within the $1500\text{--}1550 \text{ cm}^{-1}$ spectral window, enabling Raman spectroscopy to serve as a label-free probe of nitro-oxidative biochemical reprogramming (Supplemental Material, Secs. I and II H, Table S2; Fig. S4 [36]).

Raman spectra collected across NF, PF, and CF at $Q_{\min}$, Q_{ROR} , and $Q_{\max}$ ($n = 3$ independent roots per condition) capture biochemical changes that align quantitatively with the hydraulic stress indices identified in Fig. 2(d) (see also the Supplemental Material, Secs. II and IV; Table S2 [36]). Stress-associated bands in the $1512\text{--}1540 \text{ cm}^{-1}$ region intensify systematically with increasing FIS, with maximal amplitude observed under CF at $Q_{\max}$ and moderate enhancement under PF at Q_{ROR} . This spectral window is consistent with vibrational modes associated with nitro-substituted aromatic groups (NO_2 symmetric and asymmetric stretching) and S–NO bonds characteristic of nitrosothiols, both established markers of reactive nitrogen species accumulation under oxidative stress conditions [65,66]. The direction- and hydraulic stress index-dependent amplification of these bands therefore provides a label-free, molecular-level correlation of nitro-oxidative reprogramming.

The Raman spectra in Fig. 4(b) show condition-dependent differences among PF, CF, and NF conditions, indicating that biochemical responses depend not only on flow rate but

also on the spatial distribution and magnitude of hydraulic loading represented by τ_{ROI}/E . The spectroscopic signatures thus align with structural and hormonal metrics, supporting a multiscale coupling between localized hydraulic stress indices and redox-associated metabolic adaptation.

Tissue nitrogen content measured by the TKN method after 12 h further reflects these transitions [Fig. 4(c)]. Under PF, uptake increases steadily with flow rate and normalizes within the growth-permissive window [Fig. 2(c)]. CF shows an initial rise but declines sharply as flow approaches $Q_{\max} = 1.20 \text{ ml h}^{-1}$, whereas NF exhibits the lowest uptake due to diffusion-limited transport [67]. Thus, tissue nitrogen content is highest within the PF-associated optimal hydraulic loading range and declines substantially under CF conditions characterized by elevated hydraulic stress indices.

When integrated with structural and hormonal datasets, nitrogen uptake trends align closely with the mechanically defined regimes. Within the PF window, cortical architecture remains balanced [Fig. 3(e)], auxin levels remain stable [Fig. 4(a)], Raman signatures are low to moderate [Fig. 4(b)], and nitrogen uptake is maximal [Fig. 4(c)]. In contrast, under CF at $Q_{\max}$ ($\text{FIS} \approx 0.165 \text{ N m}^{-2}$), nitrogen uptake declines sharply, coinciding with reduced cortical cell area, elevated auxin accumulation, and intensified nitro-oxidative signatures. These coordinated shifts indicate that metabolic and transport capacity deteriorate in parallel with structural and biochemical reprogramming once the optimal range of hydraulic stress index is exceeded.

Figure 4(d) synthesizes these observations into a conceptual framework. Under PF, distributed hydraulic loading is associated with preserved cortical organization, relatively balanced auxin levels, and efficient nutrient assimilation, corresponding to growth-favorable conditions. Under CF at elevated hydraulic stress indices, localized tip loading coincides with increased FIS and iFIS, auxin accumulation, cortical compaction, and enhanced nitro-oxidative activity. These observations suggest that concentrated hydraulic loading is associated with coordinated structural, hormonal, and metabolic responses that differ substantially from those observed under PF.

The results described above show that nitro-oxidative signatures align with mechanically defined hydraulic stress indices, indicating that directional hydraulic loading is associated with coordinated structural, hormonal, and metabolic responses during root development.

IV. DISCUSSION

This work establishes a quantitative mechanical framework linking directional hydraulic loading to root developmental responses. By integrating the P-ROOT platform with fluid-structure interaction modeling, we demonstrate that externally imposed flow generates surface and internally transmitted stresses whose *spatial distribution* defines direction-specific hydraulic stress indices. These indices are associated with distinct growth responses, structural remodeling patterns, and biochemical adaptations under directional flow conditions. Framed through the dimensionless hydraulic stress indices τ/E and τ_{ROI}/E , hydraulic forcing becomes a mechanically

quantifiable input that can be compared across diverse flow configurations and, potentially, across species.

A key result is the order-of-magnitude separation between the optimal τ_{ROI}/E ranges associated with enhanced root growth under PF and CF conditions. This divergence suggests that the spatial distribution of stress and the steepness of local stress gradients may be more important than bulk hydraulic loading in shaping root growth responses. The results further indicate that distributed and tip-localized loading environments are associated with distinct developmental outcomes despite being generated by the same fluid medium.

This gradient-based interpretation is consistent with current understanding of plant mechanotransduction, in which localized mechanical cues regulate signaling processes, including Ca^{2+} influx and cytoskeletal reorganization. In roots, the apex represents a structurally sensitive region where geometric curvature and material heterogeneity may amplify local stresses even when average tissue loading remains relatively small. Within this framework, counterflow may act as a mechanical focusing condition by increasing stress localization near the root tip, thereby creating conditions favorable for enhanced mechanosensitive signaling.

The structural, hormonal, spectroscopic, and physiological responses observed here indicate a coordinated response to directional hydraulic loading rather than a simple monotonic dependence on flow rate. Under CF conditions characterized by elevated hydraulic stress indices, cortical compaction, auxin accumulation, enhanced nitro-oxidative signatures, and reduced nitrogen uptake were observed concurrently. In contrast, PF conditions associated with distributed loading maintained larger cortical cells, relatively stable auxin levels, and higher nutrient assimilation. These observations are consistent with a trade-off between mechanical reinforcement and transport-related functionality under concentrated hydraulic loading.

The correspondence between localized stress amplification and multiscale biological responses suggests several mechanistic hypotheses for future investigation. One possibility is that apex-localized mechanical loading is associated with modulation of Ca^{2+} -dependent signaling pathways that influence cytoskeletal organization and PIN-mediated auxin transport, ultimately affecting cell-wall remodeling and tissue architecture. However, direct experimental validation of these mechanistic links remains an important objective for future studies.

Expressing hydraulic forcing through τ/E and τ_{ROI}/E provides a transferable framework for comparing heterogeneous mechanical perturbations, including compaction, impedance, and touch, that influence root growth and adaptation through distinct mechanical environments. More broadly, the framework generates experimentally testable predictions: (1) manipulating apex geometry or local stiffness should shift the CF-associated hydraulic stress index range more strongly than the PF-associated hydraulic stress index range; (2) flow configurations that redistribute loading away from the apex should improve growth at fixed volumetric delivery rates; and (3) species- or mutant-dependent differences in elastic modulus and mechanosensitive signaling may correspond to distinct τ_{ROI}/E ranges associated with growth responses.

Although the strong correspondence between FSI-predicted stress distributions and experimentally measured structural, hormonal, spectroscopic, and physiological responses supports a mechanochemically coupled interpretation of root adaptation, the present study does not directly establish causal relationships among the individual components of this response network. Instead, it identifies direction-dependent hydraulic stress indices that quantitatively predict developmental responses across multiple biological scales. Future studies employing targeted perturbation of mechanical properties, mechanosensitive signaling pathways, and hormone transport processes will be necessary to establish the causal mechanisms through which localized hydraulic loading influences root development.

Finally, these results have direct implications for precision agriculture. In soils, infiltration and drainage generate direction-dependent hydraulic loading environments, meaning that identical nutrient delivery rates may produce different developmental outcomes depending on flow orientation relative to the root-growth axis. In hydroponic and vertical-farming systems, optimizing flow orientation in addition to flow rate may improve nutrient-use efficiency while minimizing mechanically associated growth trade-offs.

V. CONCLUSION

This work establishes directional hydraulic loading as a quantifiable mechanical factor influencing early root development. Using the hydraulic stress index τ_{ROI}/E , we show that root growth depends not only on flow rate but also on flow direction. Under both parallel flow and counterflow, root elongation is associated with distinct optimal ranges of τ_{ROI}/E . These growth-associated ranges differ by approximately one order of magnitude, with PF sustaining enhanced growth across a broader hydraulic loading range than CF.

Outside these direction-specific ranges, roots exhibit coordinated structural, hormonal, spectroscopic, and physiological responses, including cortical remodeling, auxin accumulation, enhanced nitro-oxidative signatures, and reduced nitrogen uptake. The results further indicate that these responses are more closely associated with localized stress amplification near the root apex than with bulk hydraulic loading alone.

By identifying τ_{ROI}/E as a transferable hydraulic stress index, this work provides a quantitative framework linking directional hydrodynamic forcing to multiscale root responses. While these associations support a mechanochemically coupled interpretation, future perturbation studies will be required to establish the underlying causal mechanisms.

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DATA AVAILABILITY

The data that support the findings of this article are not publicly available upon publication because it is not technically feasible and/or the cost of preparing, depositing, and hosting the data would be prohibitive within the terms of this research project. The data are available from the authors upon reasonable request.

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