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Supplemental Information

Positioning the Root Elongation Zone Is Saltatory and Receives Input from the Shoot

Tobias I. Baskin, Simon Preston, Ellen Zelinsky, Xiaoli Yang, Melissa Elmali, Dimitrios Bellos, Darren M. Wells, and Malcolm J. Bennett

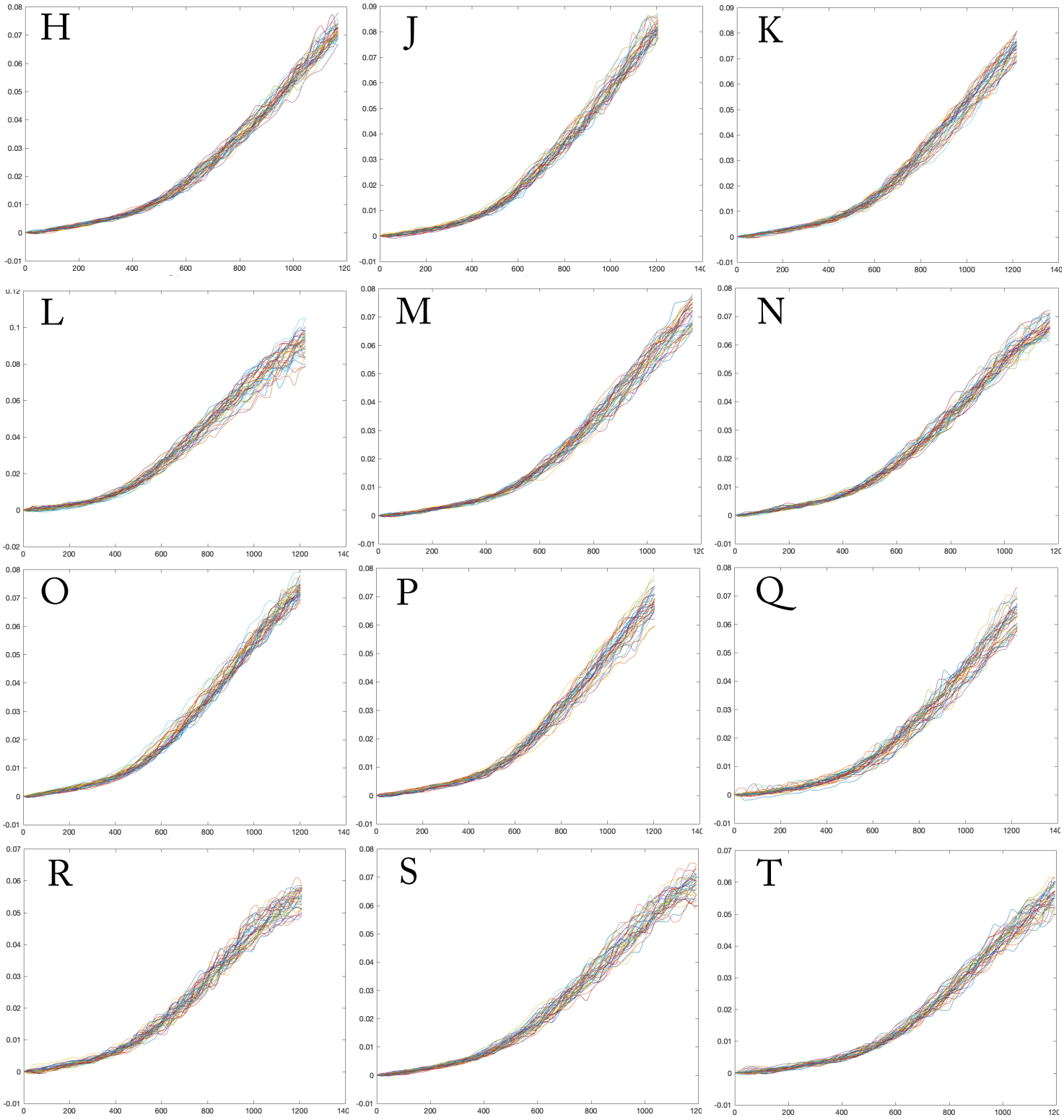

Figure S1. Superimposition of velocity profiles for intact roots. Related to Fig. 2A. Each panel shows data from a single root, imaged to yield a velocity profile every 5 min for 3 hours (37 profiles in total). All 35 of the intact seedlings are shown, identified by letter. Part 1 of 3, Nottingham roots.

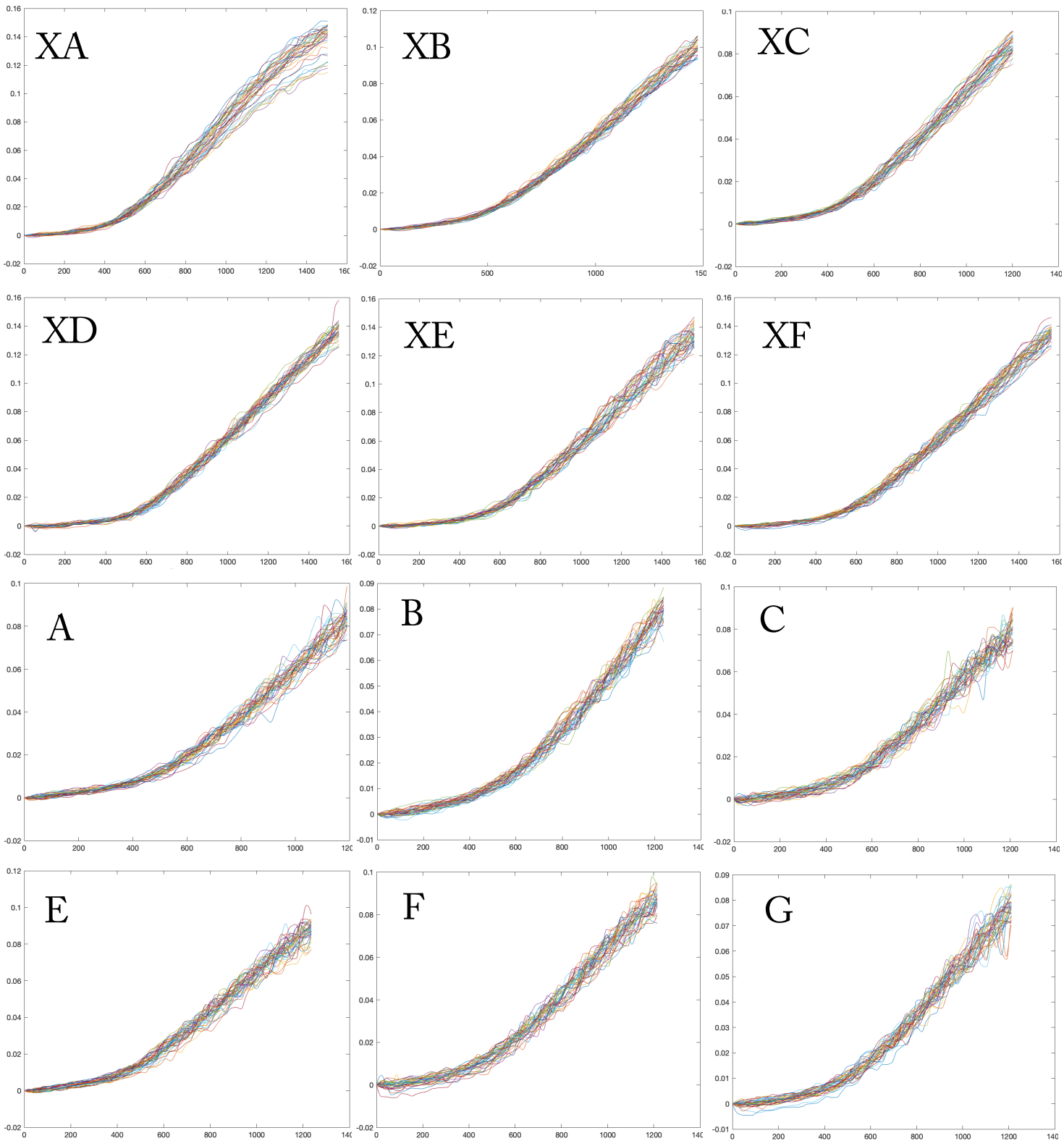

Figure S1. Part 2 of 3, Amherst roots.

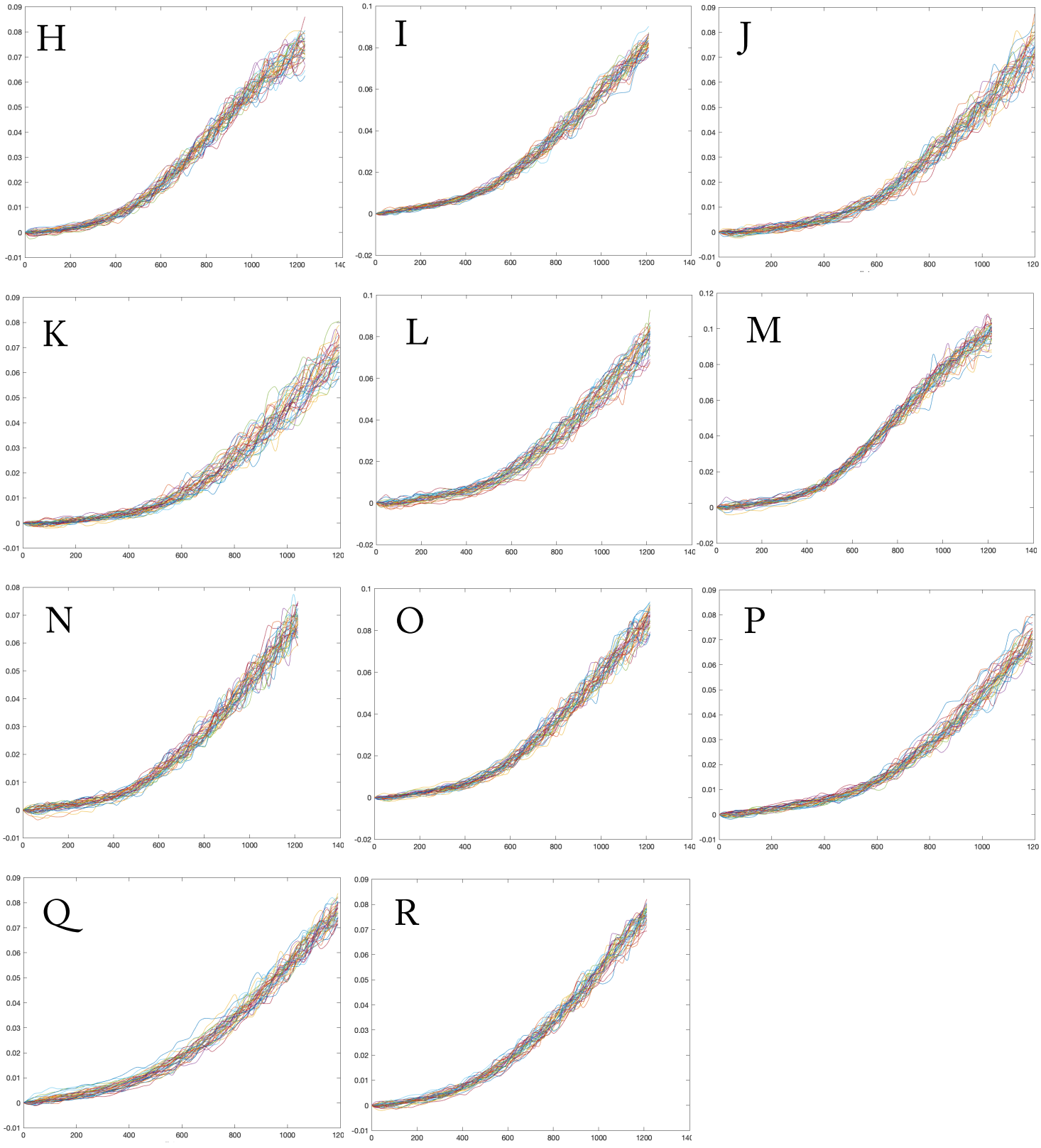

Figure S1. Part 3 of 3, Amherst roots.

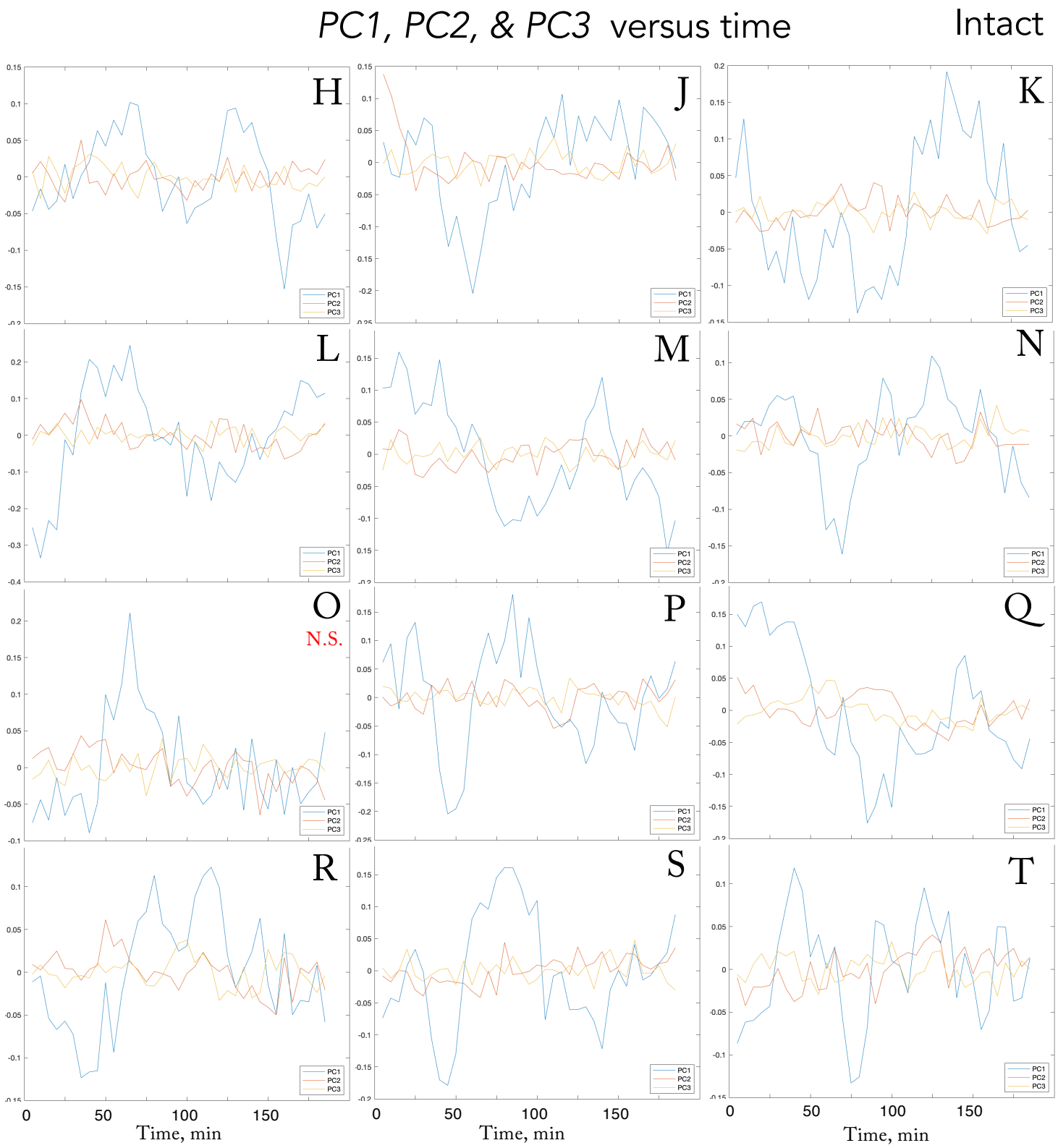

Figure S2. The first three principal components versus time plotted for each of the 35 intact roots, identified by letter. Related to Fig. 3B. N.S. indicates not significant in the runs test shown in Fig. 3A. Part 1 of 3, Nottingham roots.

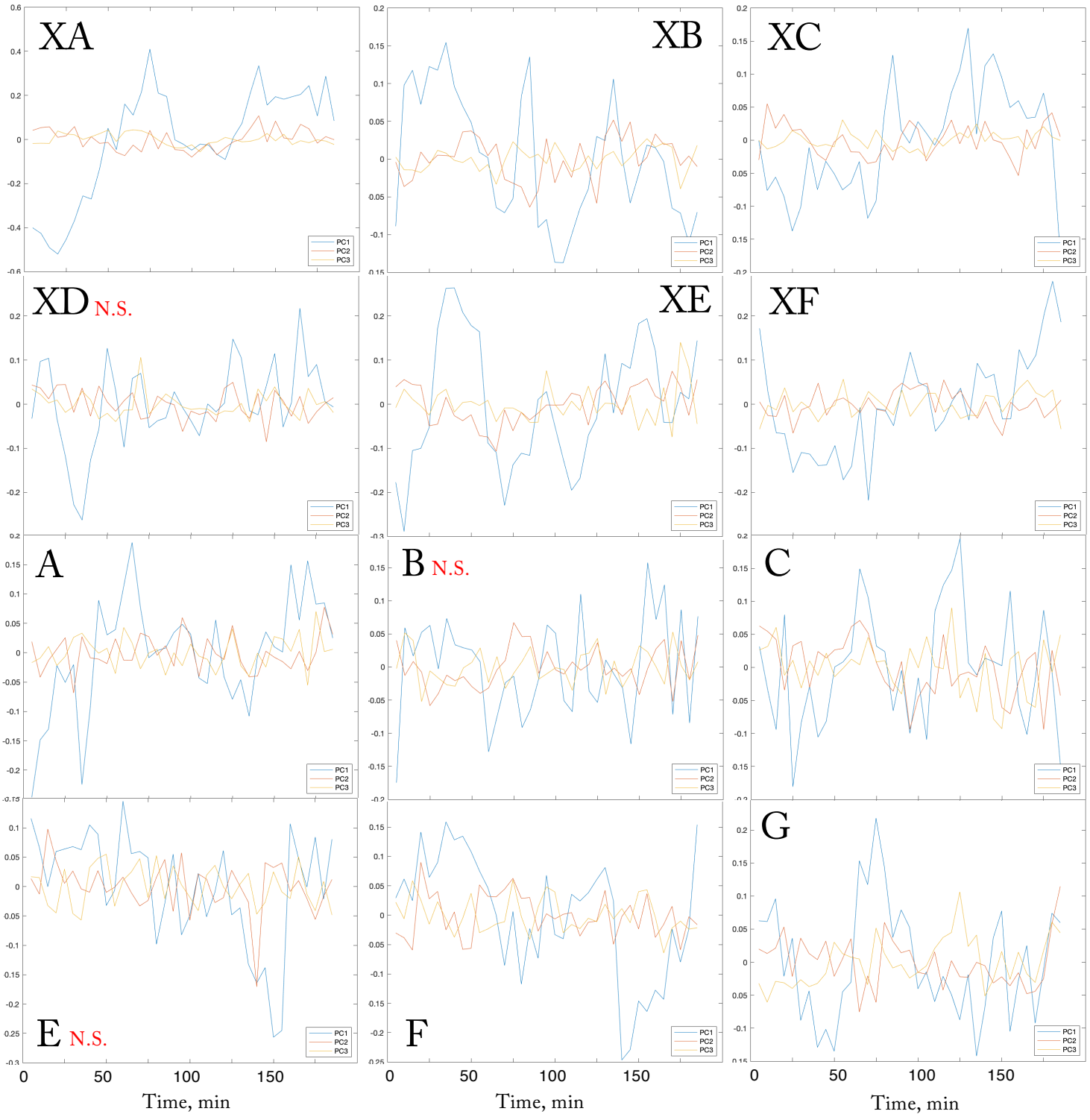

Figure S2. Part 2 of 3, Amherst roots.

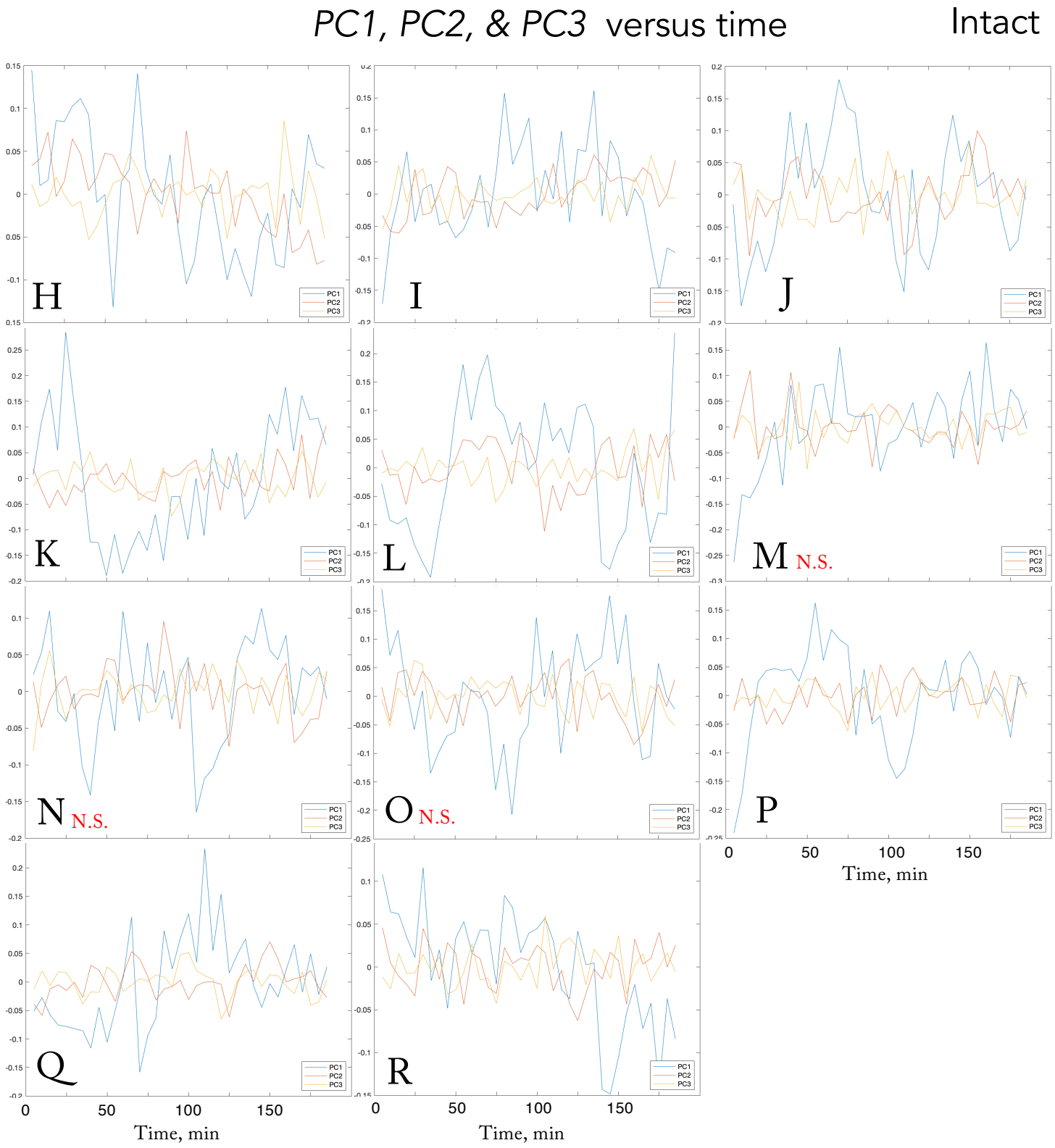

Figure S2. Part 3 of 3, Amherst roots.

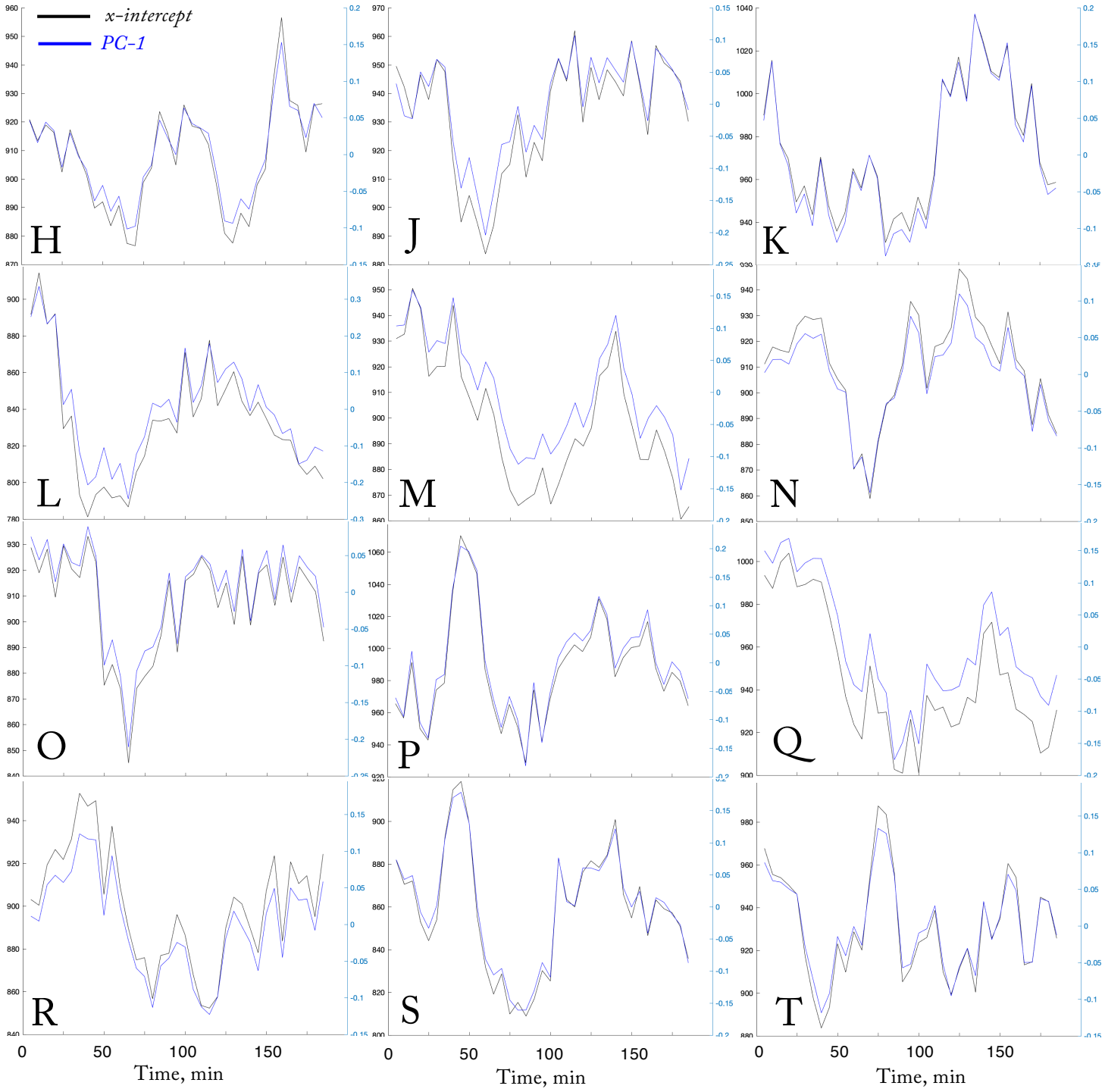

Figure S3. Comparison of the time course for principal component 1 and *x-int* for each of the 35 intact roots, identified by letter. Related to Fig. 6. For some roots, the PC1 scores have been inverted (the sign of the score is arbitrary). Part 1 of 3, Nottingham roots.

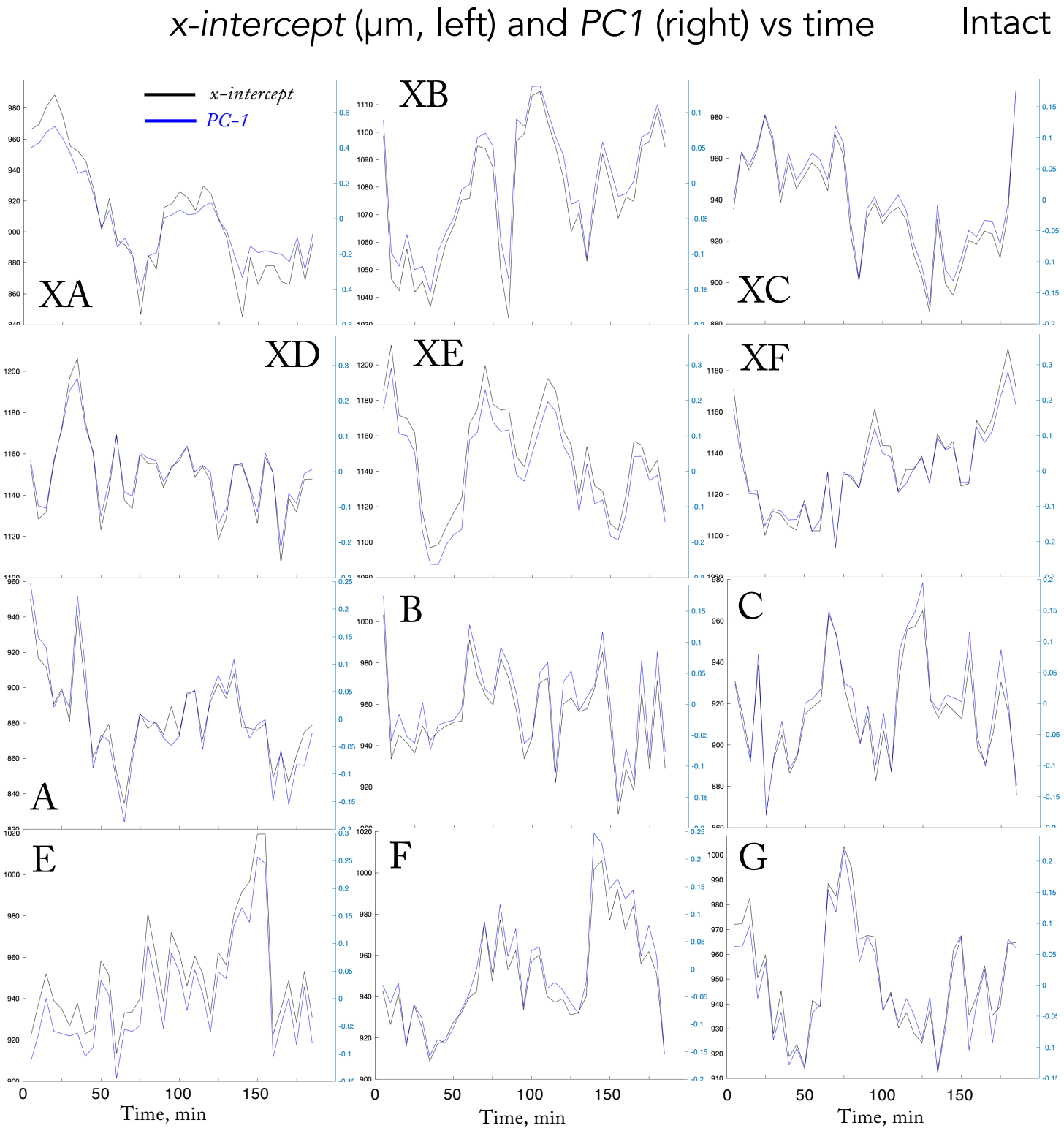

Figure S3. Part 2 of 3, Amherst roots.

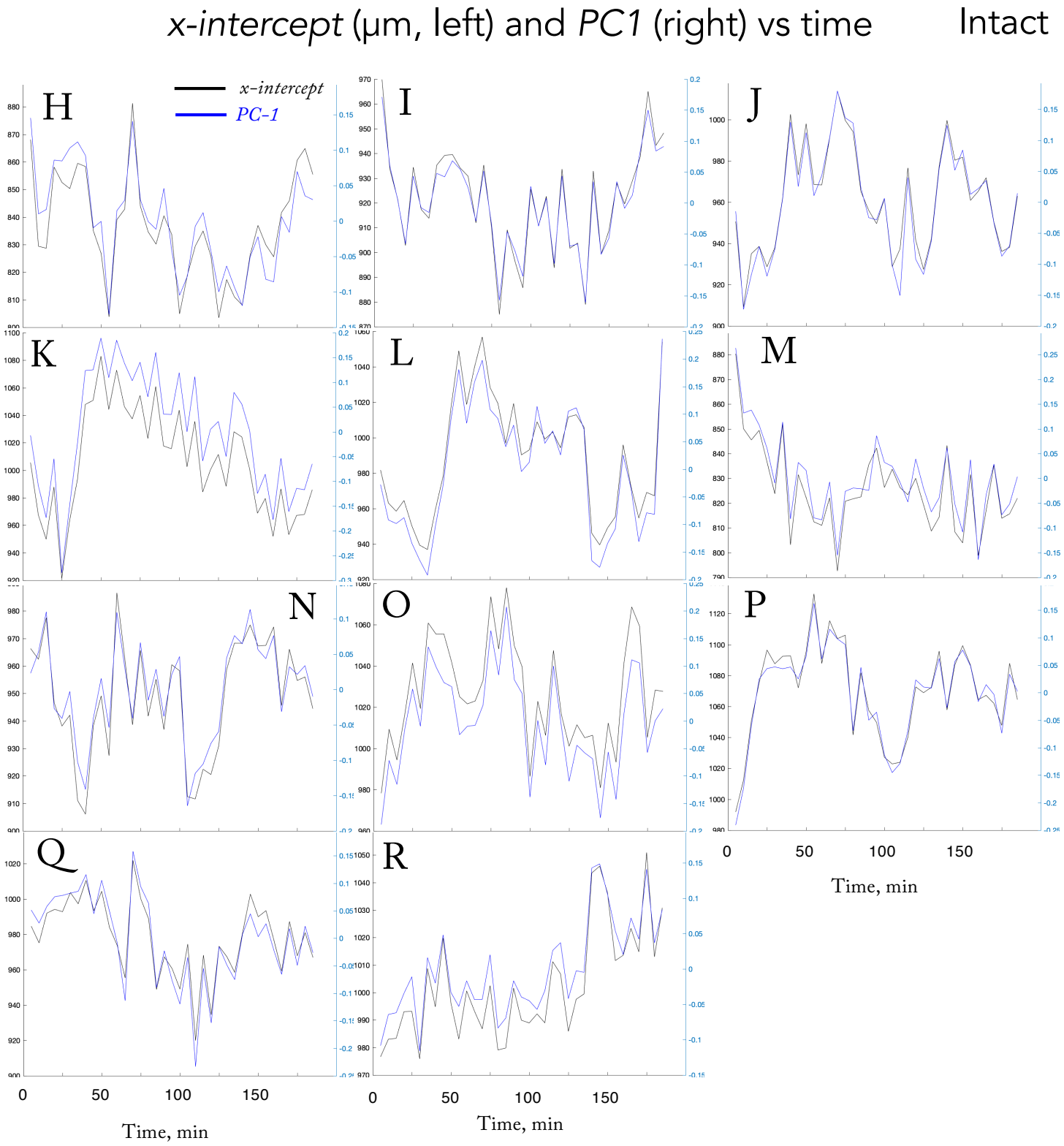

Figure S3. Part3 of 3, Amherst roots.

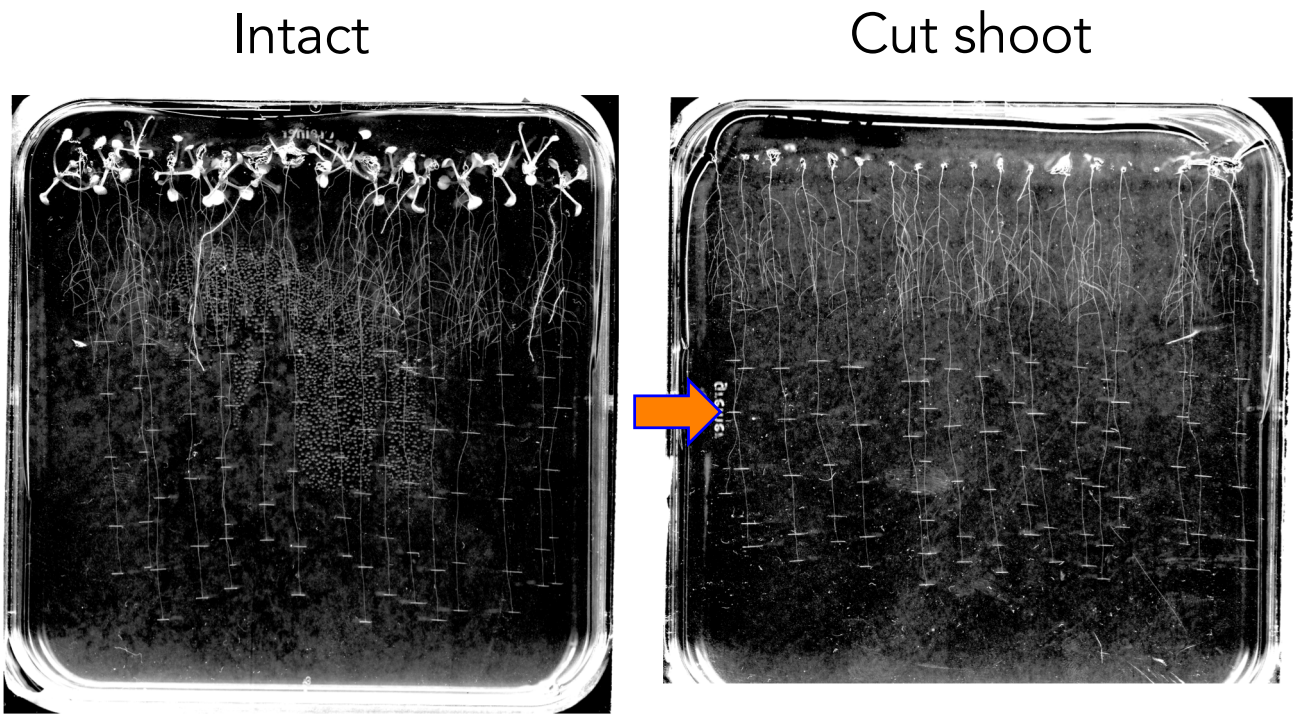

Figure S4. Roots grow for several days following shoot removal. Related to Fig. 7. To illustrate, the back of the plate was scored with a razor at the position of the root tip, once a day for five days. At the start of the second day (orange arrow) the shoots of plants on the right-hand plate were removed. After 5 days, the plates were scanned. Roots are growing inside the agar medium.

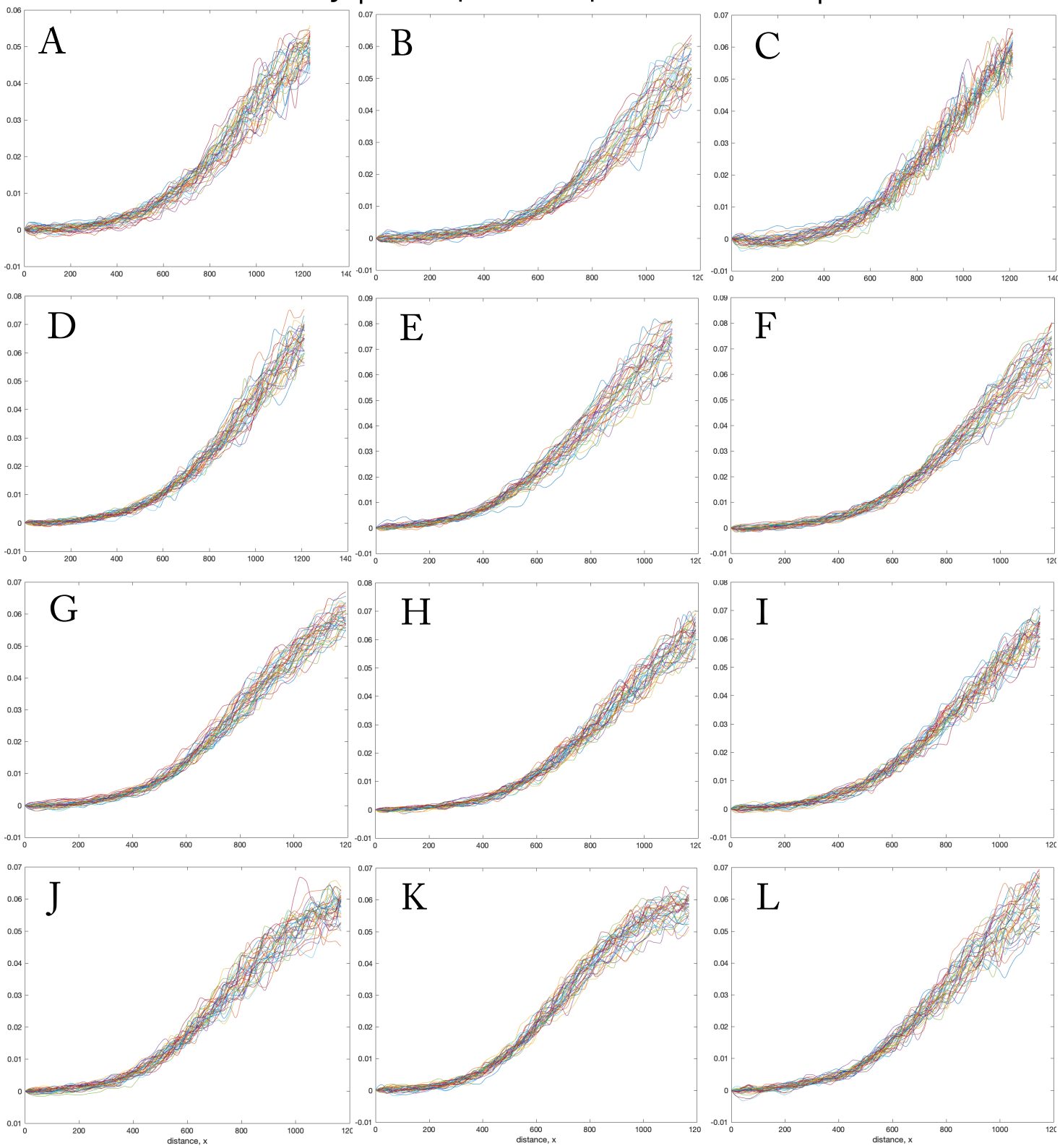

Figure S5. Superimposition of velocity profiles for roots where imaging started 2 h after shoot removal. Related to Fig. 7. Each panel shows data from a single root, imaged to yield a velocity profile every 5 min for 3 hours (37 profiles in total). All 17 of the 2 h cut roots are shown, identified by letter. Part 1 of 2.

Velocity plots: μm/s vs μm from the tip 2 h cut

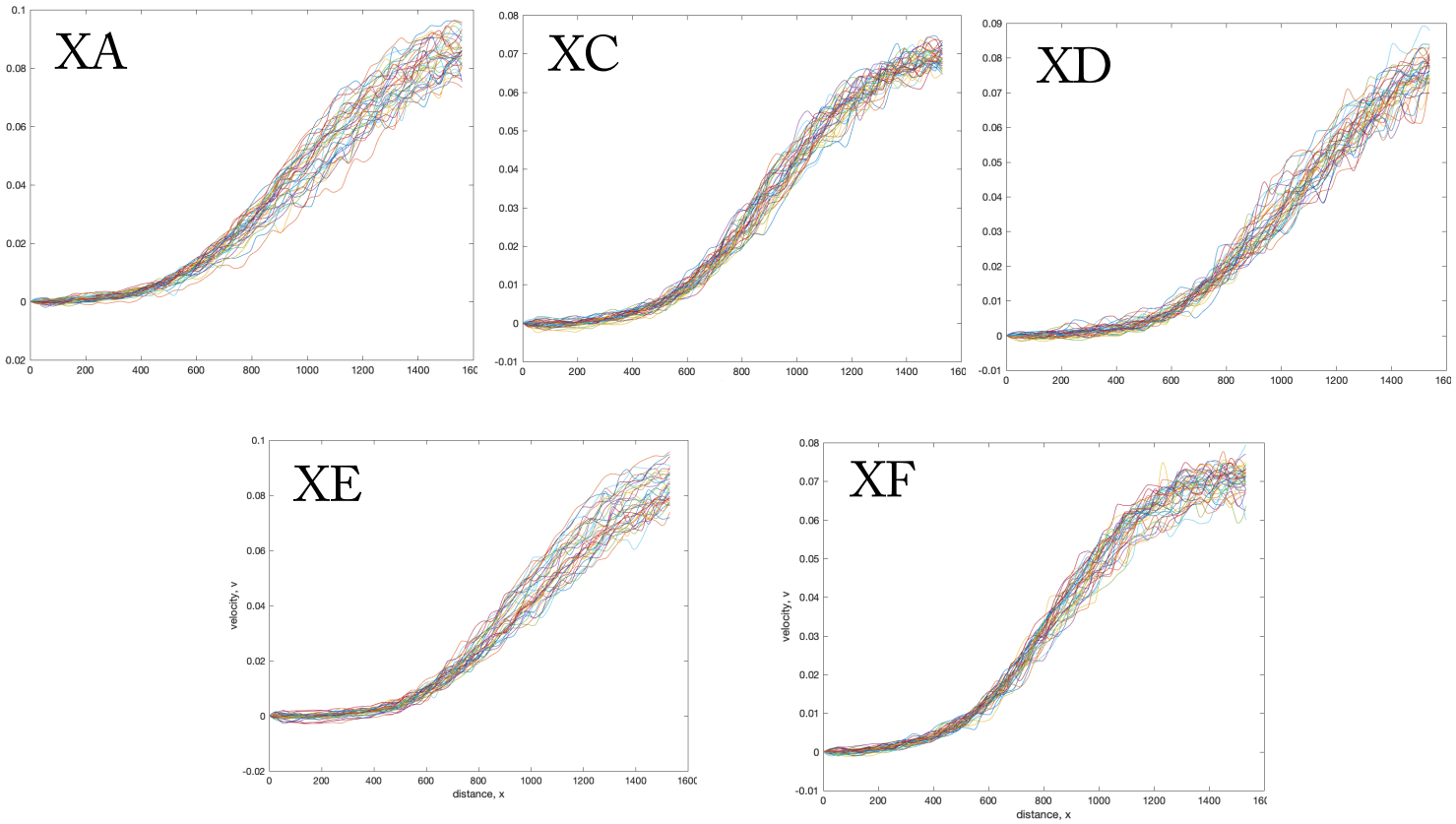

Figure S5. Part 2 of 2.

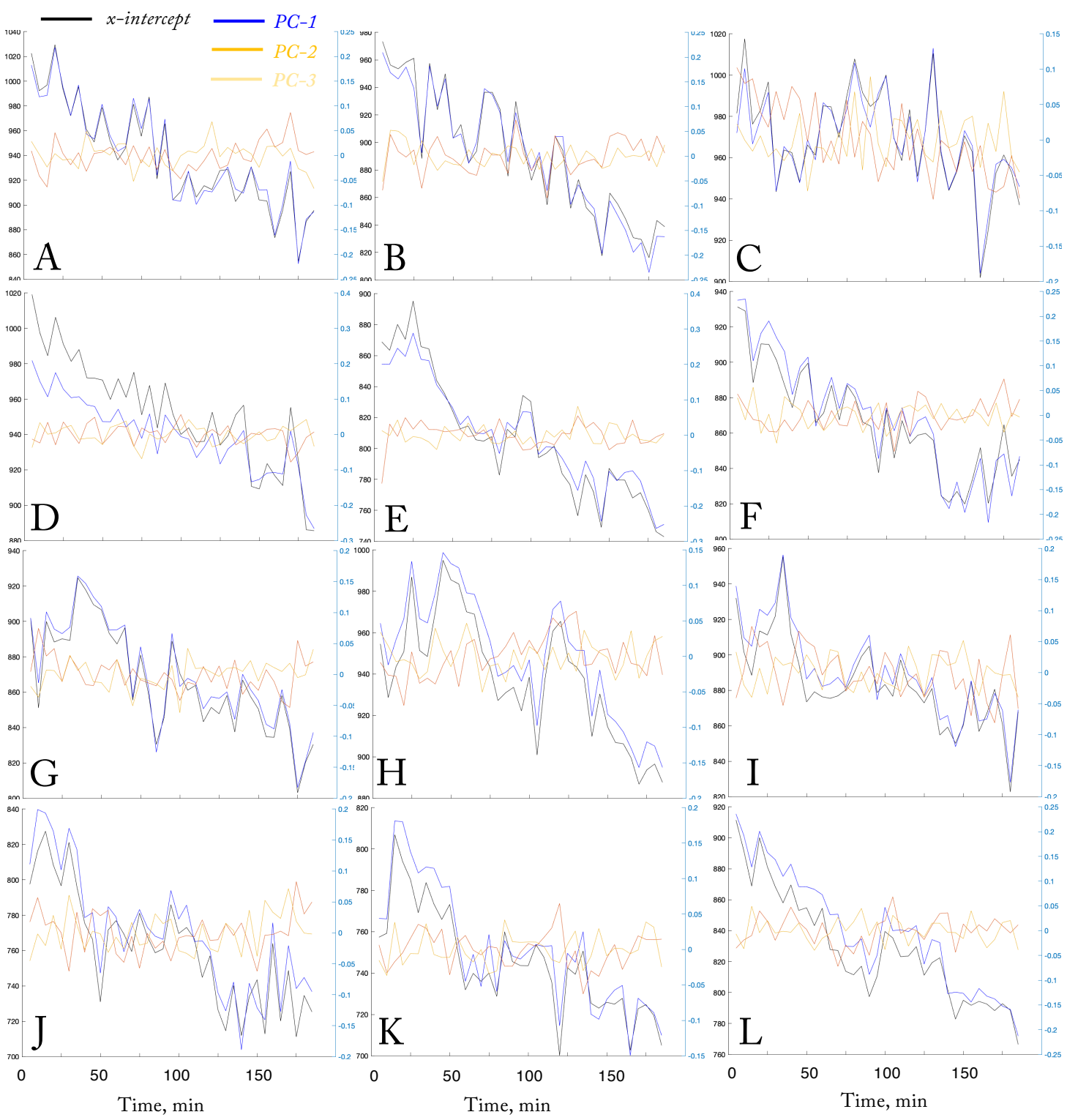

Figure S6. Comparison of the time course for the first three principal components and *x-int* for each of the 2 h cut roots, identified by letter. Related to Fig. 8. For some roots, the PC scores have been inverted. Part 1 of 2.

x-intercept (μm, left) and *PCs* (right) vs time 2 h cut

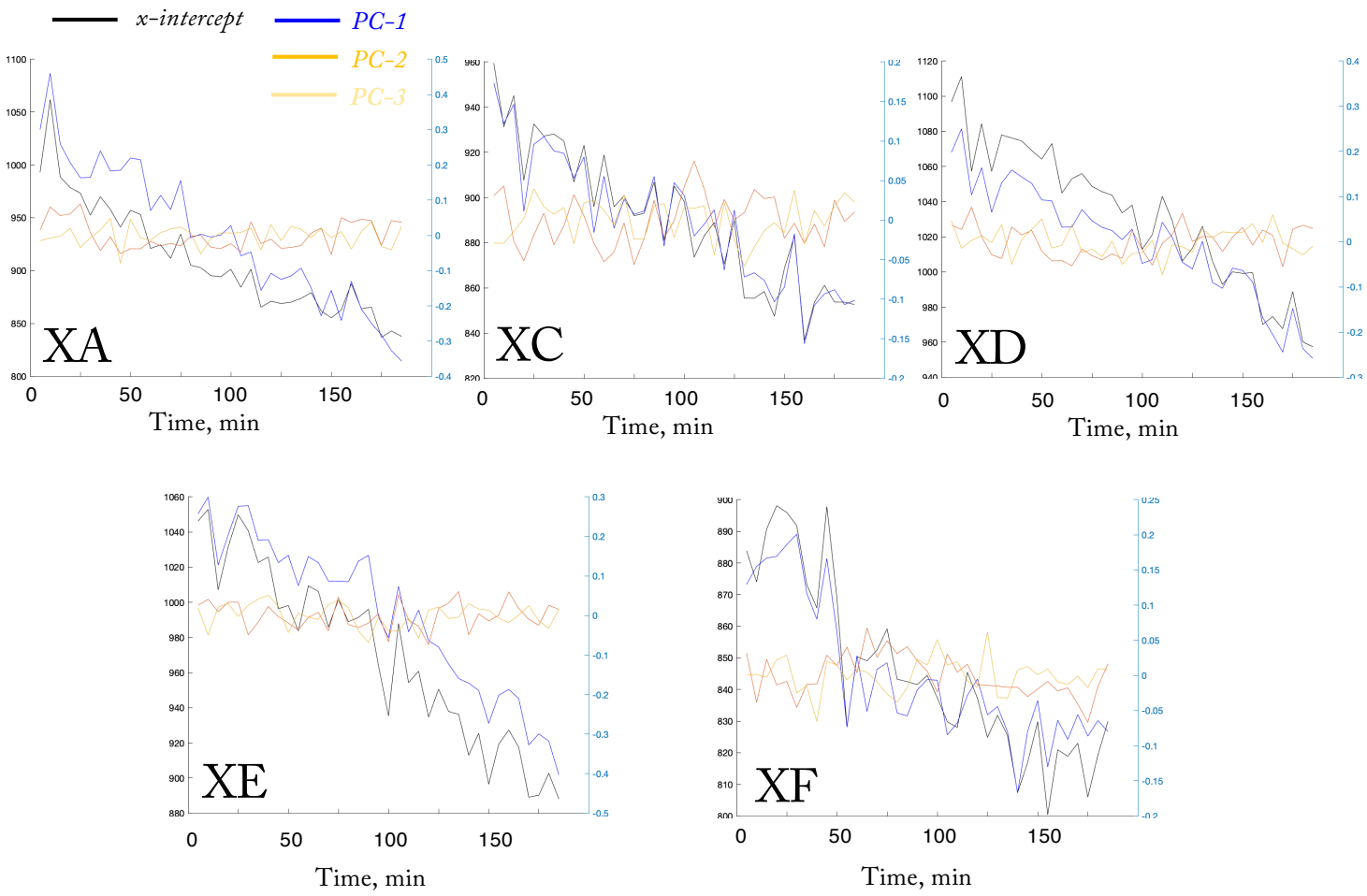

Figure S6. Part 2 of 2.

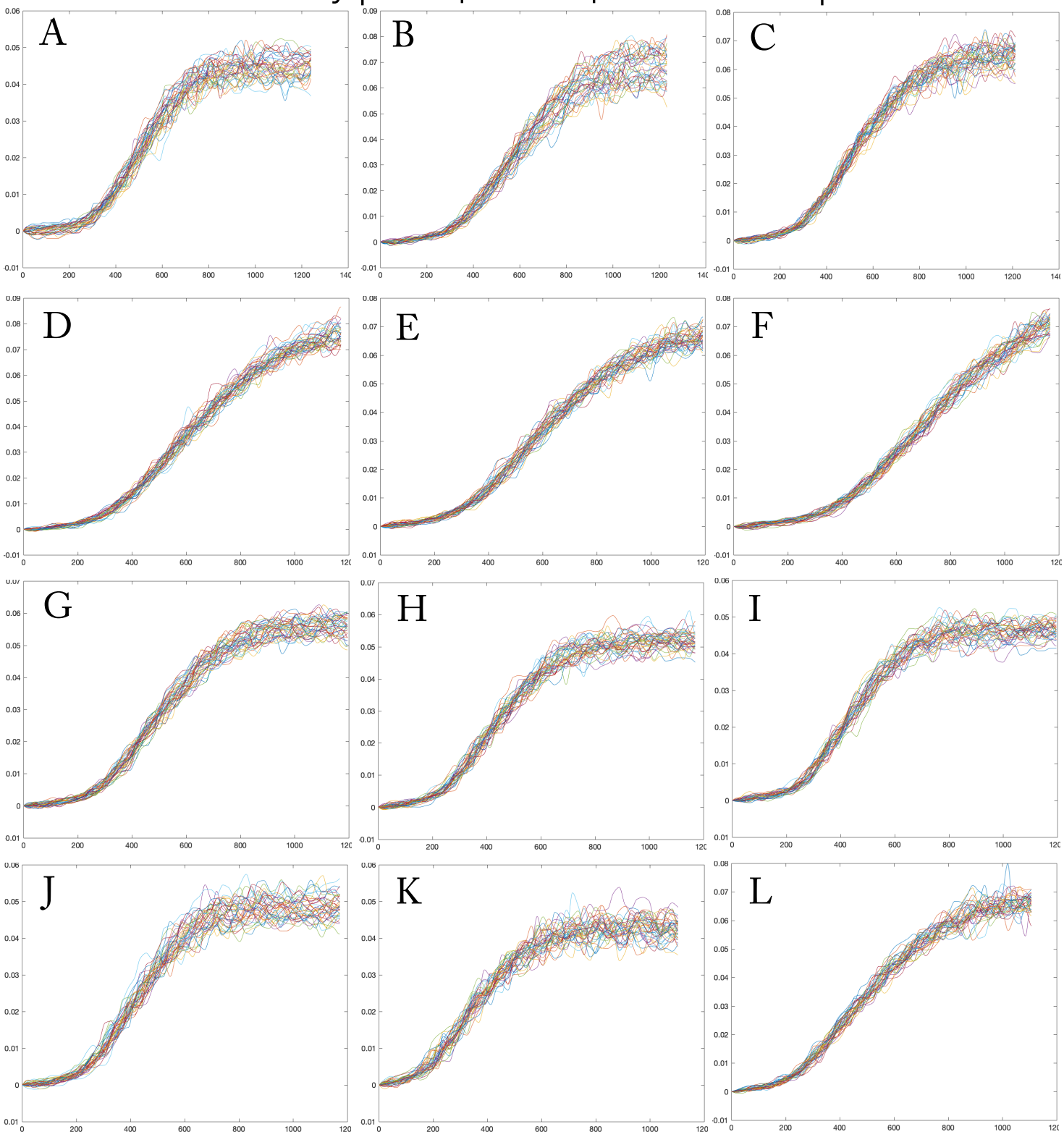

Figure S7. Superimposition of velocity profiles for roots where imaging started 24 h after shoot removal. Related to Fig. 7. Each panel shows data from a single root, imaged to yield a velocity profile every 5 min for 3 hours (37 profiles in total). All 12 of the 24 h cut roots are shown, identified by letter.

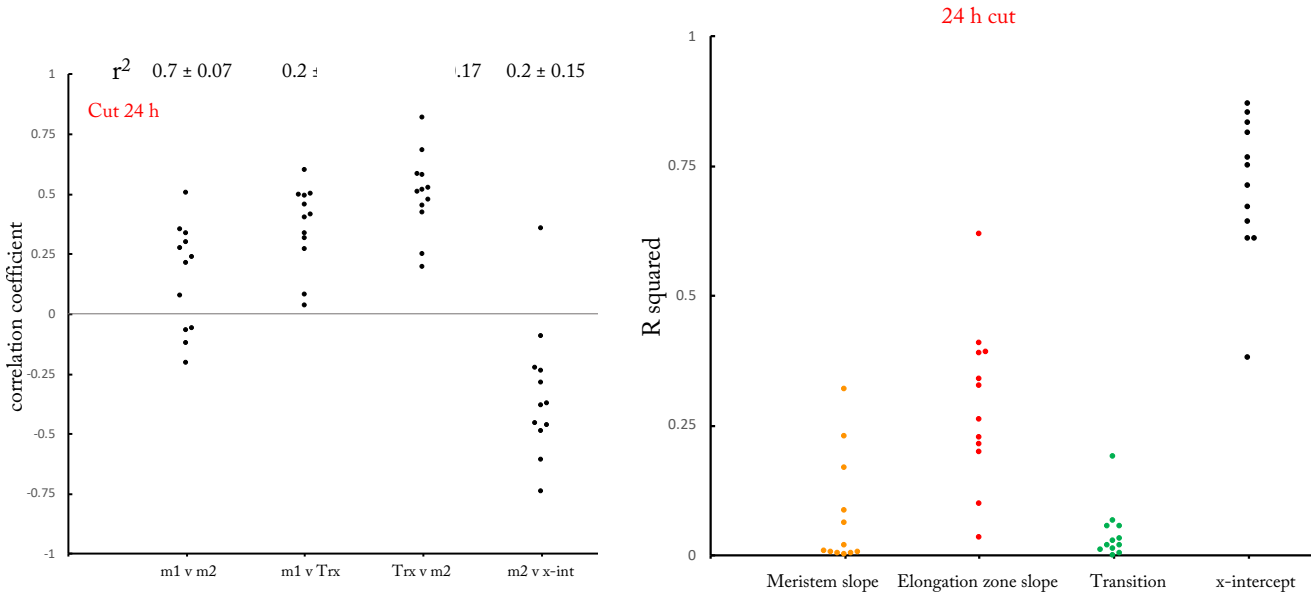

Figure S8. Correlations and time course data for roots where imaging started 24 h after removing the shoot. Related to Fig. 7 C, D. Correlation are made as for Fig. 5 A and B.

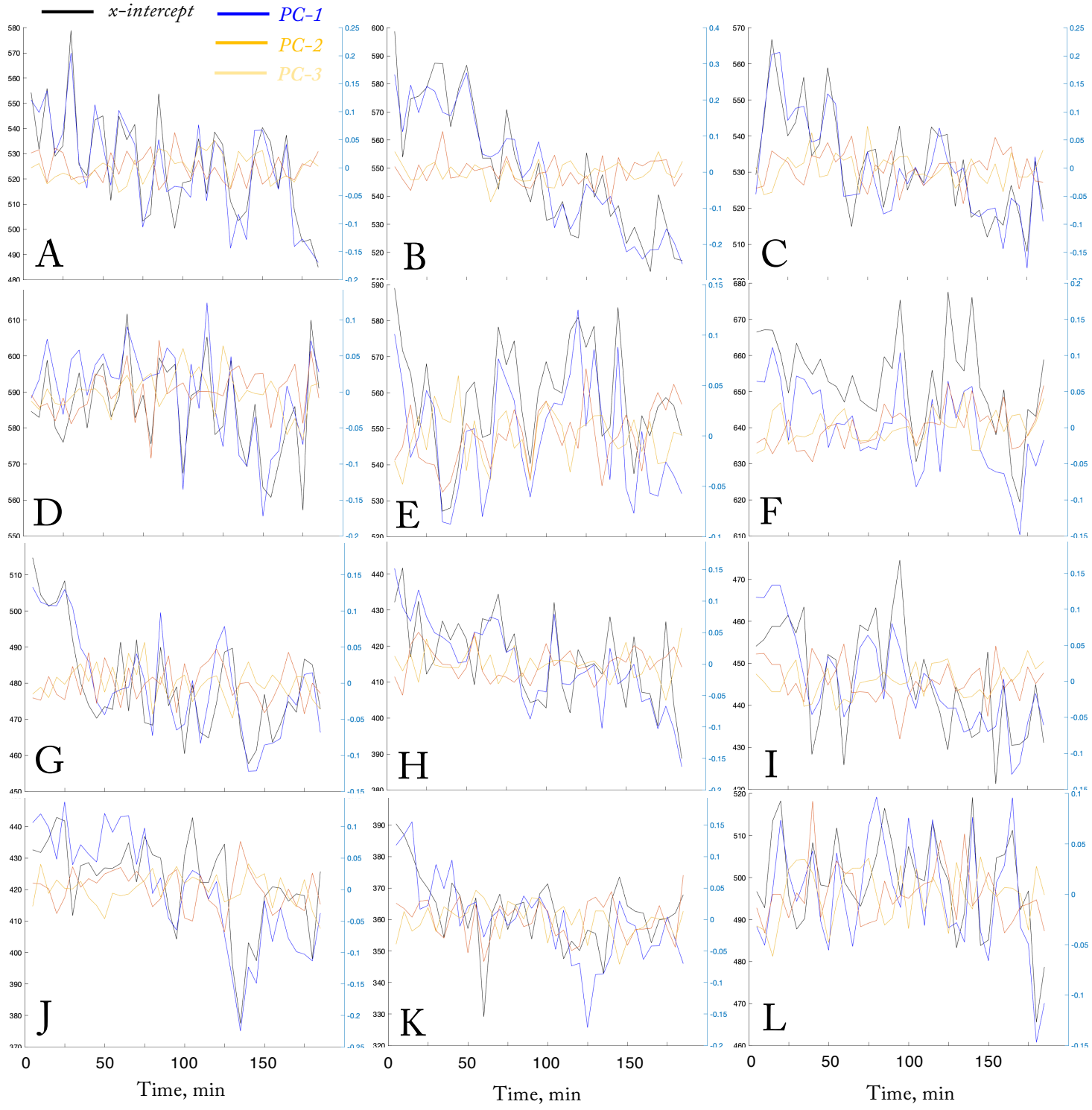

Figure S9. Comparison of the time course for three principal components and *x-int* for all 12 roots where imaging started 24 h after shoot removal, with each root identified by letter. Related to Fig's 6 & 8 and to Fig's S3 & S5.

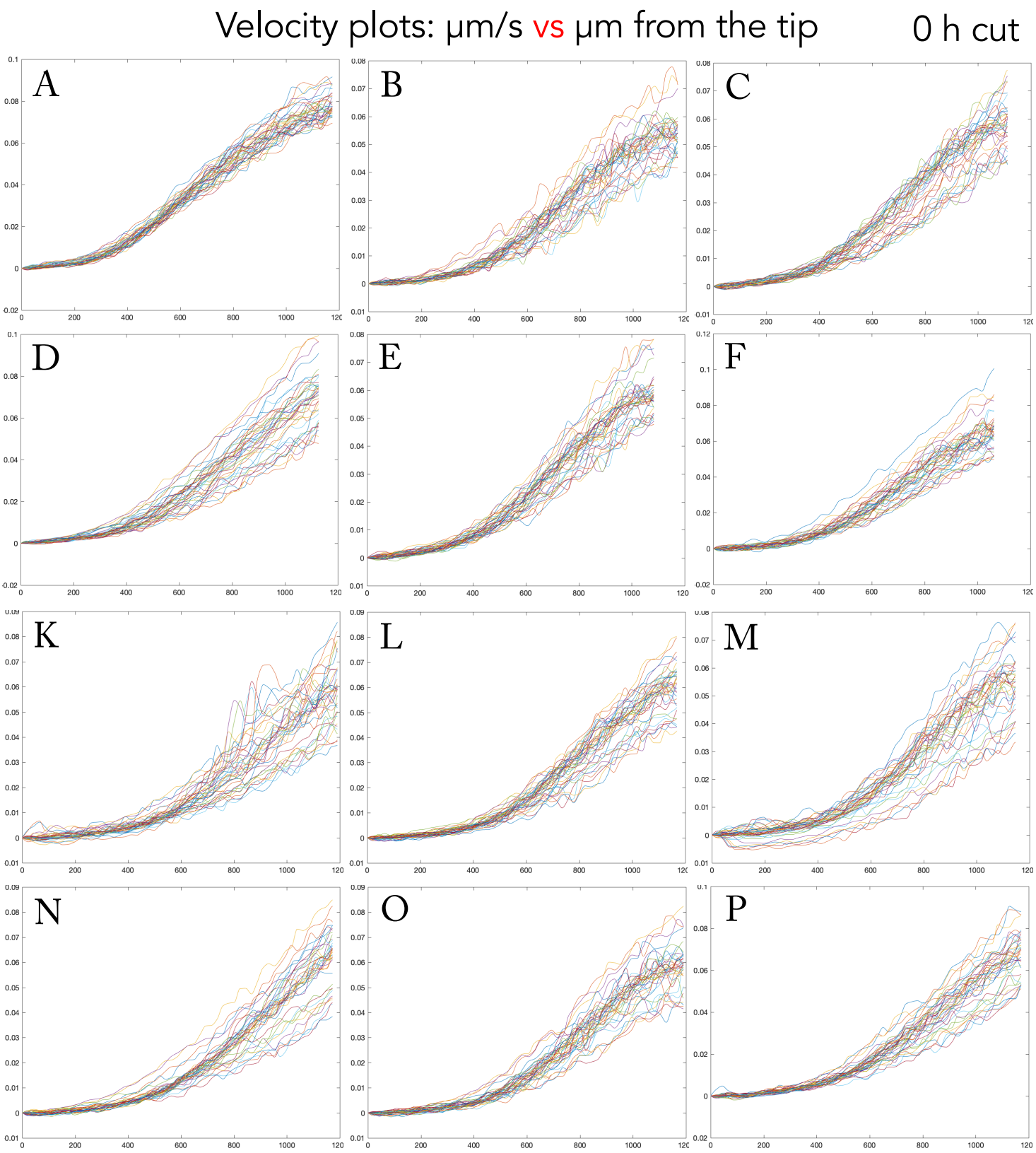

Figure S10. Superimposition of velocity profiles for roots where imaging started immediately after shoot removal. Related to Fig. 9. Each panel shows data from a single root, imaged to yield a velocity profile every 5 min for 3 hours (37 profiles in total). All 12 of the zero h cut roots are shown, identified by letter.

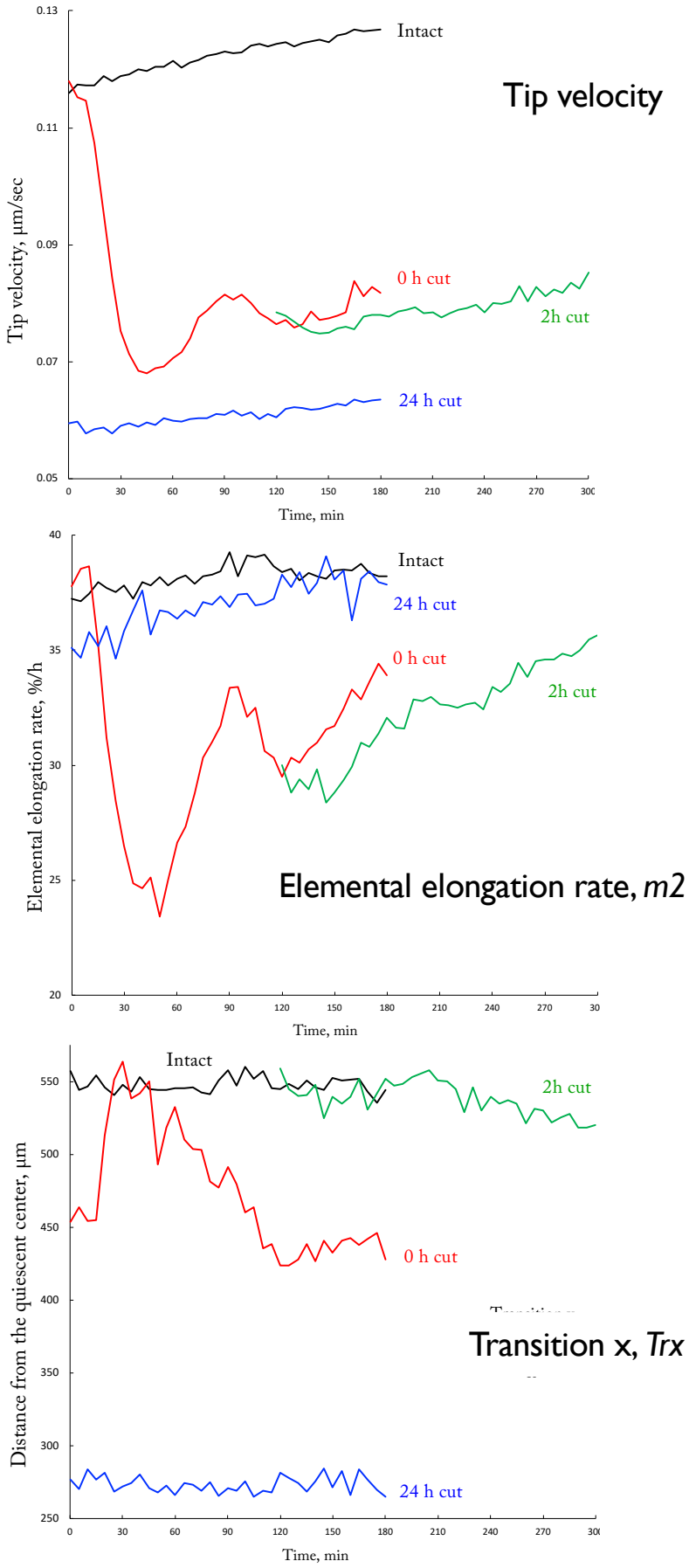

Figure S11. Comparison of growth parameter kinetics for the various treatments. Related to Fig's 4C, 7C, and 9B. Parameters are plotted as absolute values, not percentages. Data for 24 h cut are plotted as starting at time zero, for convenience.

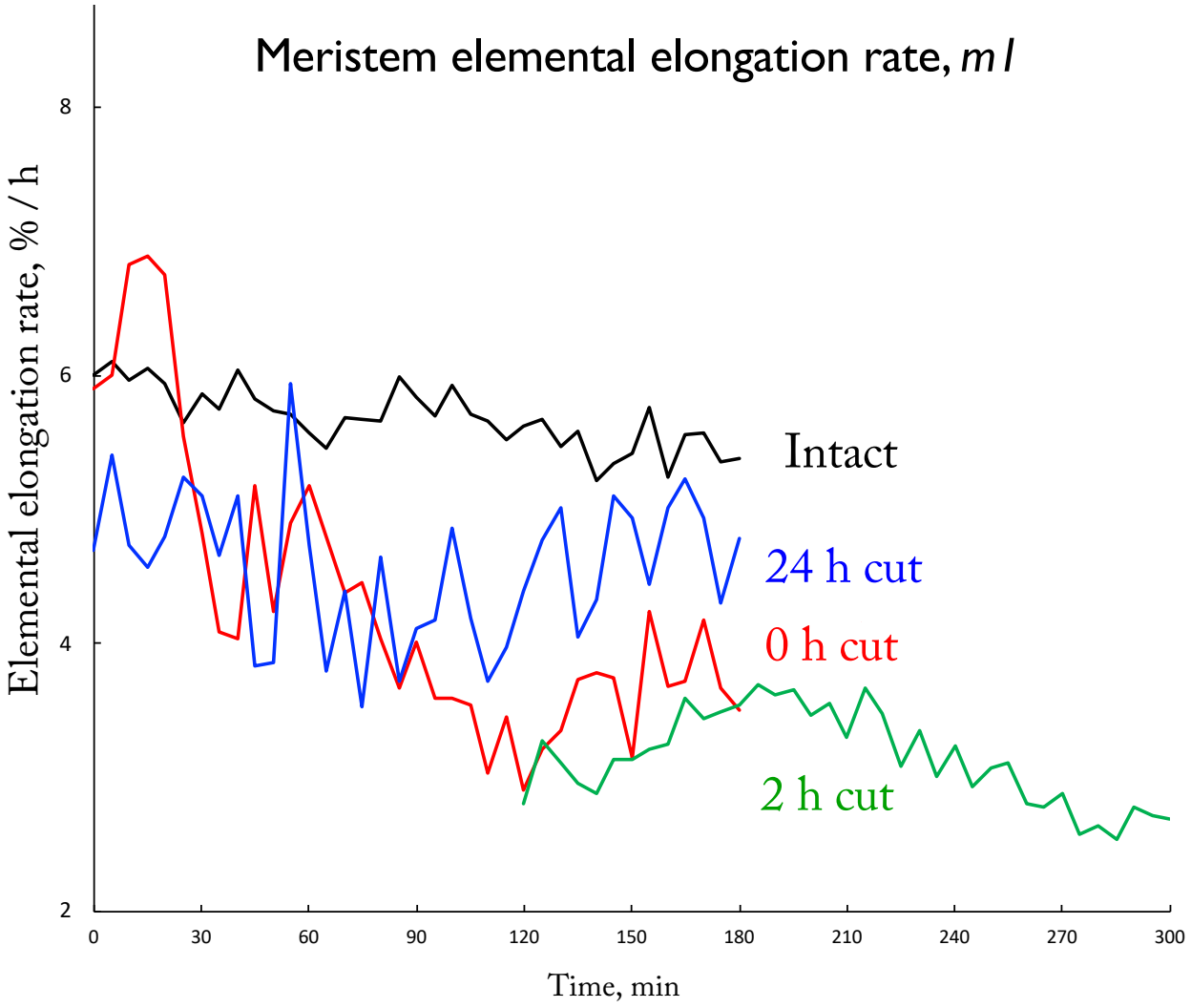

Figure S12. Elemental elongation rate for the meristem (*m1*) plotted as for Fig. S10. Related to Fig's 4C, 7C, and 9B.

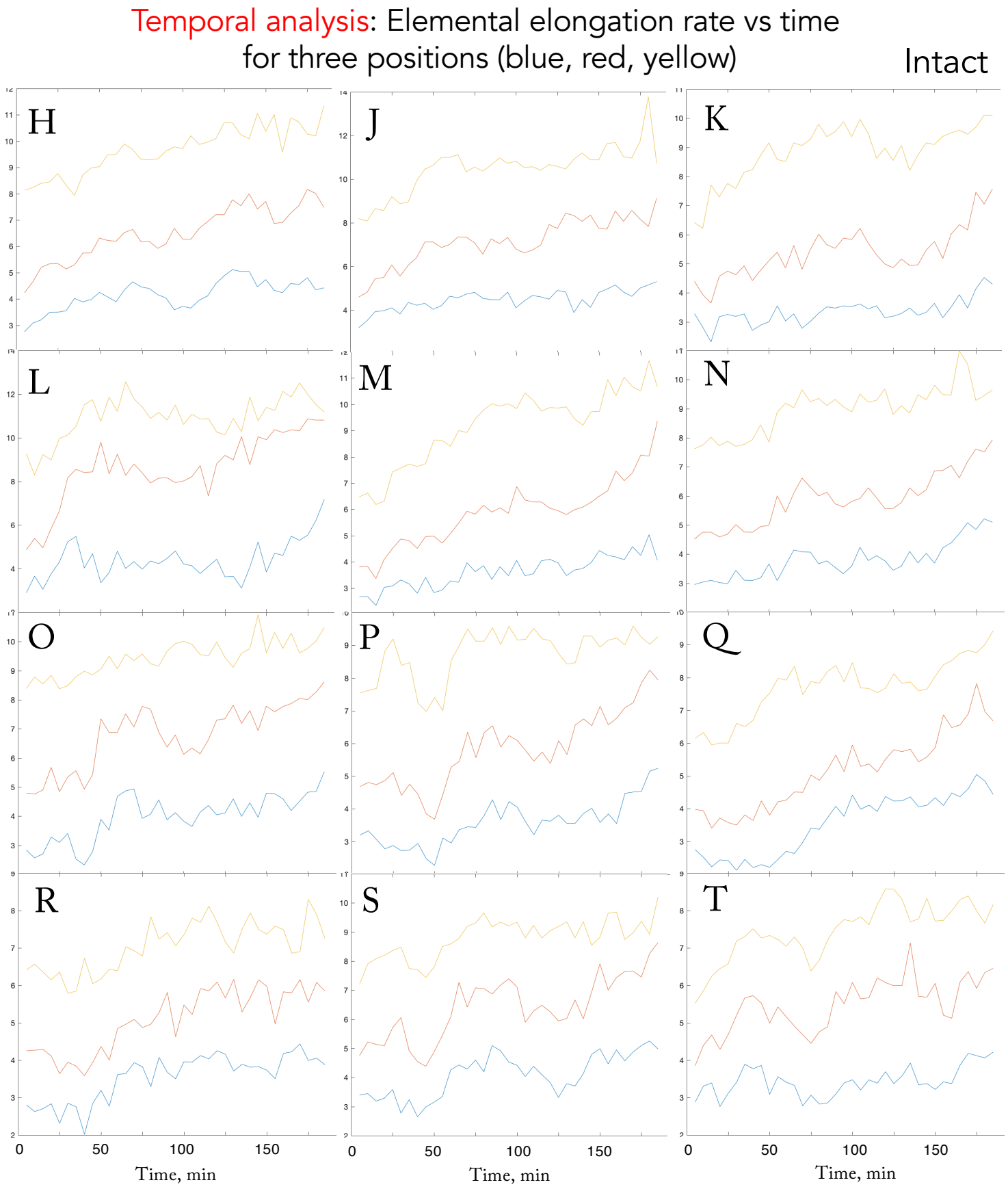

Figure S13. Temporal analysis, part 1. Related to Fig. 11B. Each panel shows elemental elongation rate of three elements plotted vs time for a single root. Results from all 35 intact roots are plotted, indicated by letter. Part 1 of 3, Nottingham roots.

Temporal analysis: Elemental elongation rate vs time
for three positions (blue, red, yellow)

Intact

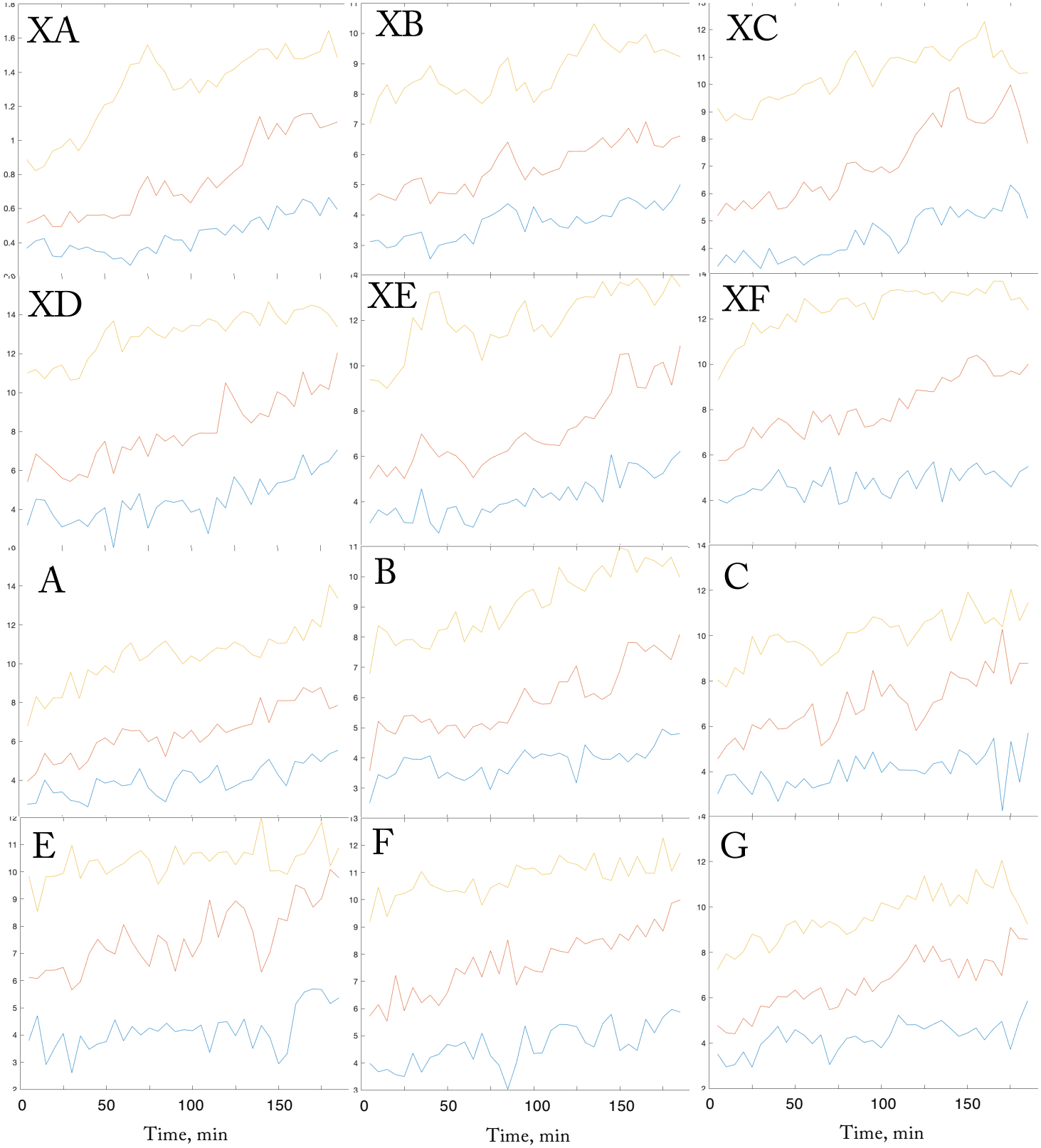

Figure S13. Part 2 of 3, Amherst roots.

Temporal analysis: Elemental elongation rate vs time
for three positions (blue, red, yellow)

Intact

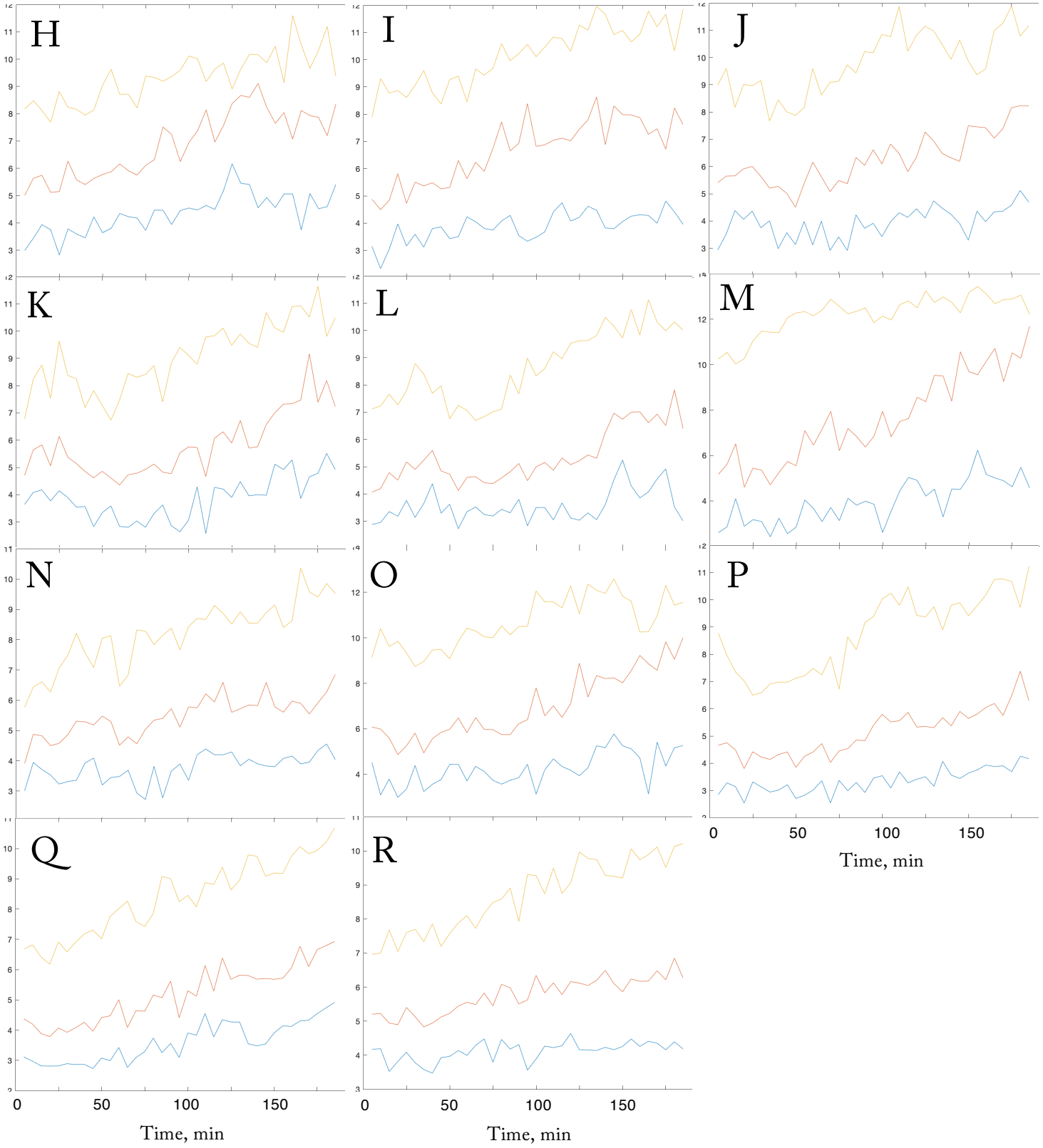

Figure S13. Part 3 of 3, Amherst roots.

Temporal analysis: Elemental elongation rate vs distance from the tip for three elements (blue, black, green)

Intact

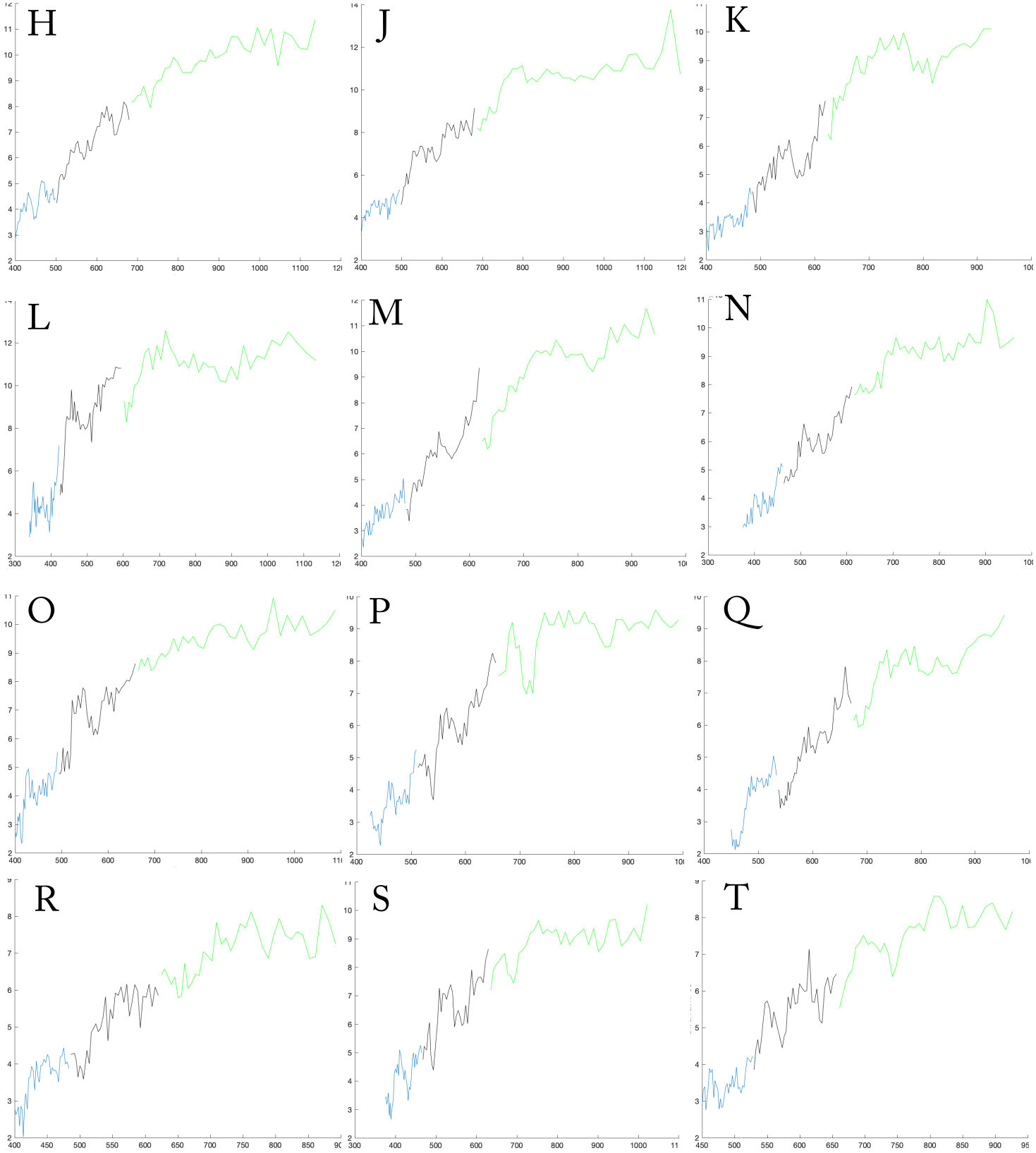

Figure S14. Temporal analysis, part 2. Related to Fig. 11C. Each panel shows elemental elongation rate of three elements plotted vs position for a single root. Results from all 35 intact roots are plotted, indicated by letter. Part 1 of 3, Nottingham roots.

Temporal analysis: Elemental elongation rate vs distance from the tip for three elements (blue, black, green)

Intact

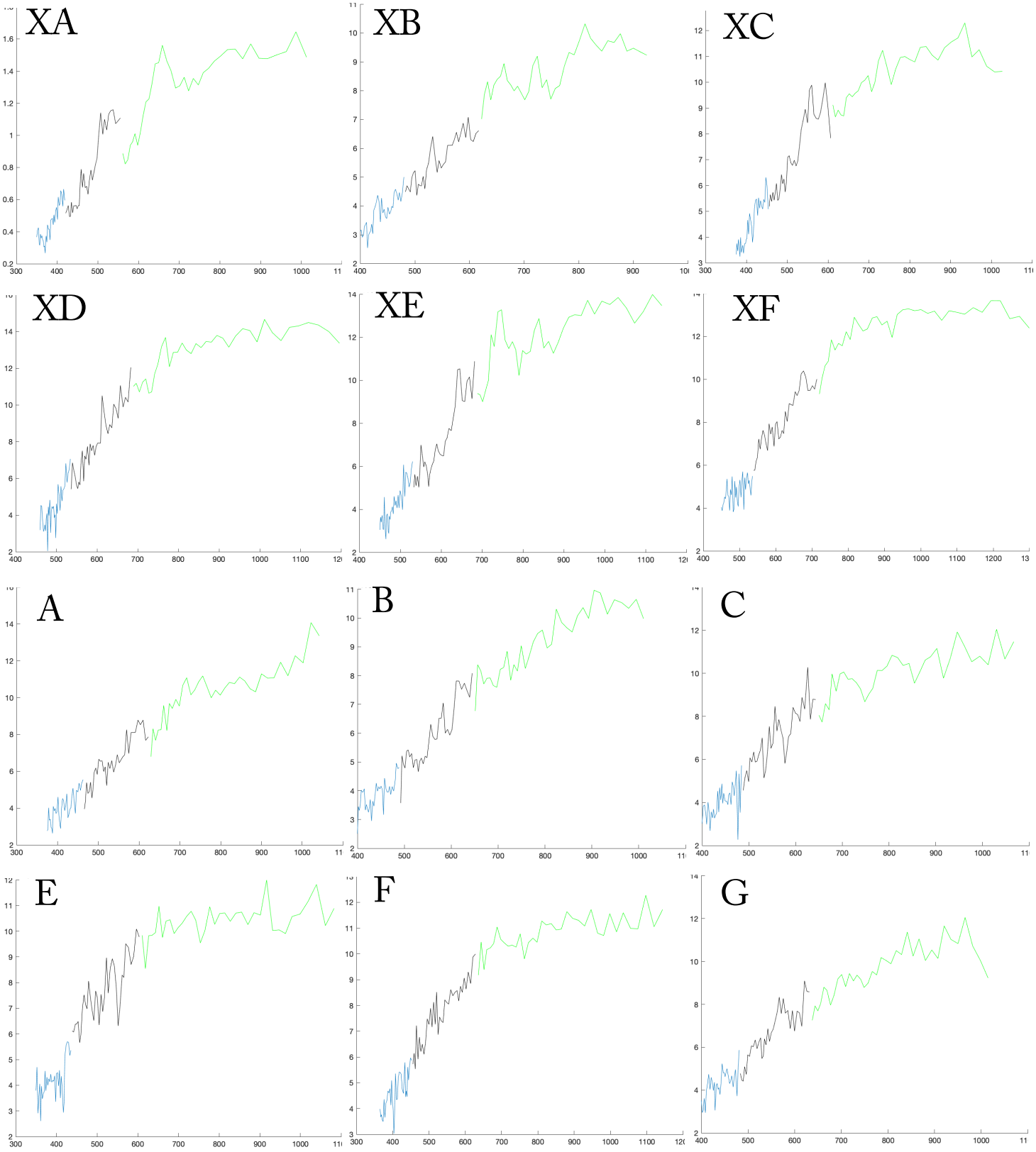

Figure S14. Part 2 of 3, Amherst roots.

Temporal analysis: Elemental elongation rate vs distance from the tip for three elements (blue, black, green)

Intact

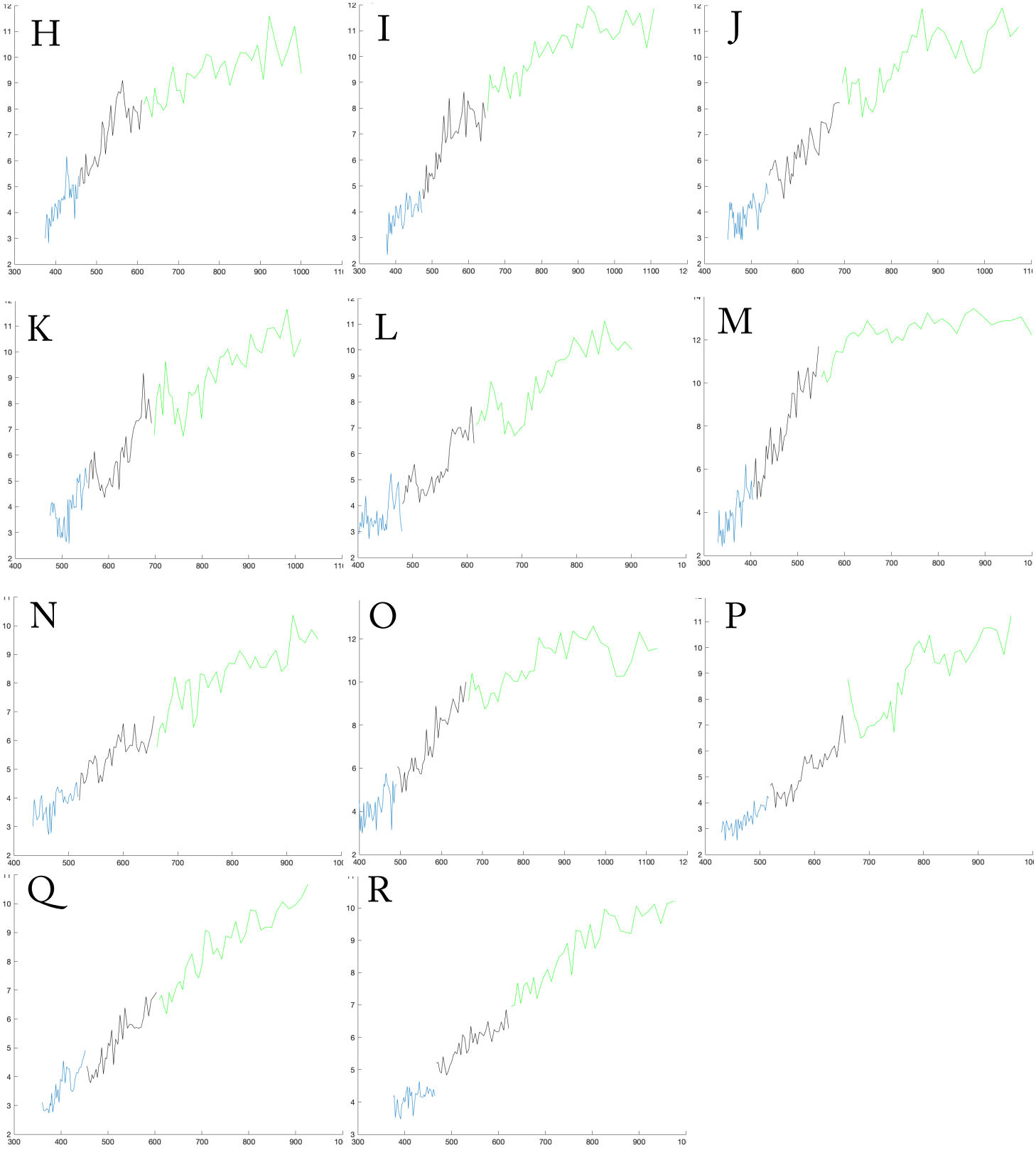

Figure S14. Part 3 of 3, Amherst roots.

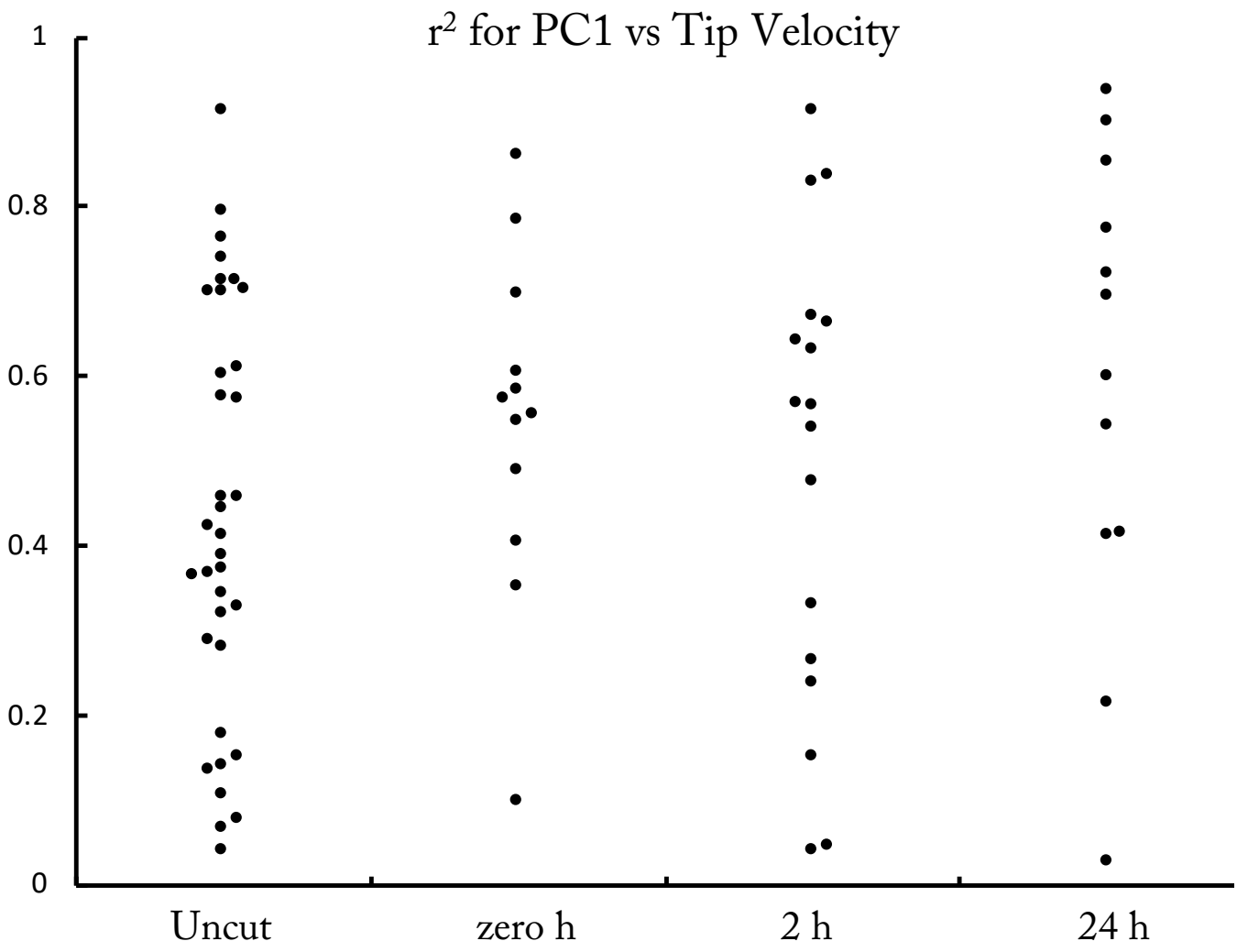

Figure S15. Correlation coefficient squared values for principal component one and tip velocity for the four treatments. Relates to Fig's 5B & D.

Transparent Methods

Plant material and growth conditions

Arabidopsis thaliana L. (Heynh), Columbia accession, seed were plated on a modified Hoagland's medium containing 0.9% agar and 1% sucrose. Composition of the medium is given by Baskin and Wilson (1997). The seeds were pushed into the agar so that following germination the roots grew inside the medium, rather than on the surface. After stratification, plates were placed vertically in a growth chamber under continuous light (100 μmol m^{-2} s^{-1}) and temperature (23˚C) and roots were imaged after 7 to 10 days. Experiments were run initially at the University of Nottingham and subsequently at the University of Massachusetts Amherst.

Root imaging and shoot excision

The plate was placed on the stage of a horizontal microscope (Nottingham: Zeiss Axiostar; Amherst: Olympus CH2) with the bottom side of the plate facing the objective. The light source was an array of infrared diodes (~900 nm). After finding a suitable root, the plate was let equilibrate on the stand for an hour (unless noted). The objective lens was 10X and the final magnification at Amherst was adjusted to match that of Nottingham by means of a variable magnification intermediate lens relay. Images were acquired via a CCD camera (Nottingham: Stingray, Allied Vision Technologies; Amherst: MicroEye, IDS Imaging) interfaced to a computer running appropriate software (Nottingham: LabView; Amherst: Micro Manager). A script was written to acquire a pair of images separated by 30 sec, wait 4.5 min, acquire a second pair of images, and so on, for 3 h (37 image pairs). From time to time during a 4.5 min interval, the microscope stage was raised manually to keep the root tip at the edge of the frame.

For some experiments, the shoot was excised. To do so, a suitable root was chosen, and then the plate opened and a fragment of a double-edged razor was used to sever the hypocotyl near the root-shoot junction and the shoot was discarded. The plate was sealed, returned to the microscope stage and either imaged immediately ("zero h cut") of after 2 h ("2 h cut"). In one set of experiments, the sealed plate was returned to the growth chamber for 24 h ("24 h cut") and then imaged after a one-hour period of equilibration on the microscope state. For the zero h cut, approximately two min elapsed between the cut and taking the first image.

For each pair of images, the velocity profile was obtained by means of Stripflow (Yang et al., 2017). This software is available here (URL1) and is described in detail by Baskin and Zelinsky (2019). Because we imaged the root for 3 h and obtained a velocity profile every 5 min, there are 37 velocity profiles per root.

Numerical analysis

For each root the velocity profiles were resampled using MATLAB's function 'interp1' so that each profile is recorded at a common set of p values of the x variable (distance), where p varied between roots but was typically around 2200. The data for each root may thus be represented as an n-by-p data matrix, with n rows and p columns, where $n = 37$ (the number of time points). Principal component analysis was performed based on the corresponding p-by-p covariance (rather than correlation) matrix. This gives rise to principal component vectors representing the primary modes of variability amongst the n profiles, and principal component scores representing the weight of the principal components for each profile (Mardia et al., 1979).

The runs test (Bradley, 1969) was performed by binarizing the temporal PC scores according to whether they were greater or less than the mean PC score and then using MATLAB's 'runstest', which tests for serial correlation in the binary sequence versus a null hypothesis that the ordering is completely random. For the temporal analysis, elemental elongation rate was obtained from the derivative of a

function fitted to the velocity profile. The function was the modified sigmoid described by Peters and Baskin (2006) truncated before the second transition. The function was fitted according to a least-squares criterion, minimized numerically using MATLAB function 'fminsearch', which is an implementation of a Nelder-Mead optimization routine.

Supplemental References

Baskin, T. I., and Wilson, J. E. (1997). Inhibitors of protein kinases and phosphatases alter root morphology and disorganize cortical microtubules. Plant Physiol. *113*, 493-502.

Mardia, K.V., Kent. J.T., and Bibby, J.M. (1979) *Multivariate Analysis: Probability and Mathematical Statistics*, Academic Press.

Peters, W. S., and Baskin, T. I. (2006). Tailor-made composite functions as tools in model choice: the case of sigmoidal vs bi-linear growth profiles. Plant Meth. *2*, 11.