Washing Roots does not Necessarily Remove Sand Implications for Pot Experiments

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In biological research it is often necessary to estimate the root mass of plants. When roots are grown in some sort of soil, it is necessary to removed clay, silt, and sand particles from roots once they are uprooted. This is often done by washing, carefully massaging out the earth from between the roots, while taking care to minimize the loss of root material. This approach works on the basic assumptions that a) any soil particles are on the outside of the roots, and b) these particles can be removed by washing.

An experiment on the response of the grass *Themeda triandra* to nitrogen, phosphorous, and potassium fertilization provided a useful opportunity to address the hypothesis that washing does not remove all sand attached to roots. *T. triandra* plants were grown in 500 ml pots for 6 months in washed river sand. This medium was chosen because it was relatively nutrient-free, and because sand is presumably easy to wash from plant roots. Plants were then removed from the pots, and separated into root and shoot fractions. A tussock grass plant consists of a number of tillers tightly to loosely joined at their bases, above whorls of seminal and adventitious roots (Briske 1991). Structurally, owing to the way the bases of the tillers are joined, it might appear that a grass plant has three sections: the leaves, a hard, almost woody base (or crown), and the roots, possibly leaving one with the dilemma of whether to cut the leaves off the root + base or the roots off the leaves + base.

The latter is the correct option if true roots are to be separated, and was the procedure used here. The roots, which were often pot-bound and filled the entire volume of the pot, were carefully and thoroughly washed, and the shoot and root fractions were dried at 60°C for 48 hours and weighed. The roots were then incinerated (ashed) at 600°C in a kiln, and the residue was weighed.

Amount of Sand in the Root Samples

The average proportion of residue in the root samples was 40.3% ± 16.0 SD, with a range from 6.70 to 74.8%. There was a significant postitive relation between root mass and residue mass ($_{F1,83}$ = 44.9, $P < 0.0001$, R^2dj = 34.3%), indicating that the larger the root mass, the more residue it holds.

The relation between root mass and the proportion of residue was not significant ($F_{1,83}$ = 1.40, P = 0.23, R^2dj = 0.47%), indicating that the proportion of residue that is attached to the roots is constant from small to large roots. The residue was not chemically analysed, but certainly had the appearance of the sand in which the plants were grown, along with a small amount of grey ash. Samples were washed to remove soluble salts and re-weighed, revealing that on average 93.8% of the residue was insoluble, presumably comprising sand and silica.

Sand and Silica in and on Plants

The presence of silica in plants, especially grasses, has been well documented (see O'Reagain and Mentis 1989), and its role as an anti-herbivore defense mechanism has been suggested. McNaughton et al (1985) argued that grasses accrue silica as a defense to large mammalian herbivores in the Serengeti. In their study the silica contents of leaf blades and sheaths of laboratory-grown grasses were 2.7 and 3.7% respectively, while roots, which had been washed in water to remove soil, had silica contents of 11.3%. Curiously, above-ground leaf litter had the highest silica content – 12.8% - which is higher than for any other part of the plant. This seems impossible (how does a plant accrue silica once it is dead?), but a possible explanation is that exogenous dust and grit accumulate on fallen material, thereby increasing its silica content. McNaughton et al (1985) did use electron microscopy to determine that the insoluble ashed material was indeed silica, but did not explicitly test whether it was plant- or soil-derived.

Sanson et al (2007), while indicating that silica is not, in fact, harder than tooth enamel, noted also that dust and grit are a likely source of silica in the forage of animals.

Implications

The results from this single study indicate that conventional root-washing methods may not be a satisfactory way of removing sand from roots (e.g. Ghebrehiwot et al (2006) used a hose and a bucket of water to wash sand from *T. triandra* roots). One way around the problem was given by Badgery et al (2005), where the problem of recalcitrant soil in root samples was addressed by ashing a subsample of the population of roots with which they were dealing, and using a linear regression between root + soil weight vs. residual soil to correct root weights of the remaining values. They found that the amount of sand was directly related to plant size, but did not give the results of the regression, or whether they felt that it was suitably accurate for use as a correction factor.

While this is certainly a move toward addressing the problem, the results from the current study suggest that it is not good enough. Although there was a significant relation between sand mass and root mass, the predictive capacity of the model is relatively low (34%). The recommendation, therefore, is to always ash root samples after they have been dried and weighed to account for trapped sand particles.

References

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