Commentary

Doing the best we can: the realities of measuring non-structural carbohydrates in trees

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As researchers, we strive for science quality, with key criteria revolving around whether our work is generalizable, repeatable, falsifiable, accurate and precise (Bartneck 2007, Chalmers 2013). Science quality is assumed to be implicit in the scientific process, with peer review the main way that quality is assessed. Failure to meet these criteria can limit the interpretation and value of our results, particularly when trying to compare across studies to help expand our knowledge of given systems. This means that there is an onus on us as researchers to adopt processes and methods that help us to meet these criteria, and to report in detail on the approaches we have used so that others can judge the quality of our work.

Seemingly small variations in methodologies can have large implications for our capacity to meet scientific quality criteria. A good example of this is studies of non-structural carbohydrates (NSCs) in trees. Non-structural carbohydrates are the building blocks of life, not only responsible for growth and survival, but also underpinning plant responses to the environment (Kozlowski 1992). There is a growing body of evidence that NSCs play a critical role in the way that trees respond to environmental stresses such as drought and defoliation, and in stress recovery (Allen et al. 2009, McDowell et al. 2013, Adams and al. 2017). Understanding of NSC pools and fluxes is also important for addressing questions about ecosystem function and plant adaptation (Ryan 2011, Dietze et al. 2014). Despite decades of research, our understanding of NSC patterns and processes is limited (Hartmann et al. 2018), and recent reviews have highlighted major gaps in knowledge, including mechanisms controlling NSC allocation, the degree to which storage competes with growth, how storage might change with growing conditions and life stage, and whether NSC storage and remobilization is an active or passive process (Martinez-Vilalta et al. 2016, Hartmann et al. 2018). For many of these issues the literature is inconclusive. Hence our capacity to represent NSCs in forest models is generally simplistic, which limits our ability to predict the consequences of global environmental change on the world’s forests (Hartmann et al. 2018).

Two recent studies have highlighted that at least some of this uncertainty arises from the methodologies used to collect, extract and quantify NSC (Quentin et al. 2015, Landhausser et al. 2018). Quentin et al. (2015) assessed the commonly published NSC methods used for woody tissues, and identified four primary extraction and five primary quantification methods for soluble sugars alone, with many laboratories developing modifications to the primary methods that significantly influenced results (i.e., there is a plethora of unique methods, all giving different results). They found that NSC concentrations for some tissues ranged from 53 to 649 mg g\(^{-1}\) for the same tissue analysed in different laboratories. Their conclusion was that absolute NSC estimates for woody plant tissues cannot be compared among laboratories because of the large amount of unexplained variation between laboratories for the same method, although a consistent use and reporting of reference standards may help to overcome this. This finding has serious implications for cross-comparisons of NSC studies. Some authors have managed this problem by focusing on relative rather than absolute differences in NSC between studies (e.g., Martinez-Vilalta et al. 2016).

The work of Landhausser et al. (2018), described in this issue, provides some welcome guidance on how to improve the
accuracy and precision of NSC measurements in trees, and how to standardize methods so that inter-laboratory comparisons are valid. Using six laboratories, they measured a common set of nine plant materials and two synthetic samples with known NSC concentrations, using the same protocols across laboratories for sugar extraction and starch digestion, and three sugar quantification methods. They also tested how factors such as sample handling affected results.

These researchers demonstrated that sample handling and storage are unlikely to be a major source of variation in results, meaning that laboratories can focus their efforts on improving protocols for extraction and quantification. This does not mean that sample handling is not important (Quentin et al. 2015), simply that some of the more problematic elements of sample handling, such as immediate refrigeration and/or microwaving of samples, are not as critical as some of the other steps. Their finding that using a standard protocol resulted in good accuracy between laboratories is critical, and suggests that it is possible to compare absolute NSC between laboratories provided a standard protocol is used by all laboratories. This is a major step forward. They have developed standard protocols for laboratories to apply for specific types of research questions and target compounds. Their results highlight the particular importance of strict protocols for the extraction of soluble sugars, starch digestion and NSC quantification, and point to this as an important focal area for laboratories undertaking NSC assessments.

Reference standards are best practice in a range of plant chemical analyses (Saunders et al. 2004), but the lack of commercially available reference standards for NSCs means they are rarely included in NSC studies (Quentin et al. 2015). Landhausser et al. (2018) provide guidance on how, in the absence of commercial reference material, the researcher community can construct its own reference standards comprising multiple NSC components (monosaccharides, disaccharides, oligosaccharides, starch). They recommend that authors use reference standards to report on the accuracy and precision of their results in all publications.

In their recent analysis on the dynamics of NSCs in terrestrial plants, Martinez-Vilalta et al. (2016) highlighted that many papers are published with insufficient details to allow inclusion in cross-study analysis. Landhausser et al. (2018) recommend that experimental meta-data should include a detailed description of the plant, its location and phenological stage, the material tested and where on the plant it was collected from. It should also include information about the reference standards used and the percentage recovery for these standards. The increasing use of and access to data repositories increases the need for appropriate meta-data, to facilitate data searches and retrieval. Numerous meta-data best practice guides are available that can help in the development of protocols (e.g., Best Practices in Creating Metadata, ICPSR, http://www.icpsr.umich.edu/icpsrweb/content/deposit/guide/chapter3docs.html; Metadata Best Practices, DataONE. http://www.dataone.org/best-practices/metadata; Metadata Services, Cornell Research Data Management Service Group, http://data.research.cornell.edu/services#Metadata).

There is a strong imperative to improve the accuracy, precision and repeatability of NSC experiments, so that the results of individual studies can be integrated to provide the generalizable and mechanistic insights required to advance our understanding of the role of NSCs in plant responses to environment. While there are differences of opinion as to whether standard protocols are an appropriate way forward for NSC studies (Quentin et al. 2015), Landhausser et al. (2018) have demonstrated that implementing standard protocols can contain measurement variability to 10% or less, resulting in improved accuracy, precision and repeatability of results within and between laboratories, which should in turn improve science quality. By providing such clear evidence of the benefits of applying standard protocols, and offering clear guidance on appropriate methods to apply, there is a strong case for standardization that was more difficult to justify before this study. Some of the negatives remain, such as the costs of refitting a laboratory to accommodate standard methods and the potential loss of connectivity with data collected using prior methods. It is ultimately up to the scientific community to decide whether the potential benefits outweigh the costs. In making that decision, the impact of not changing on science quality, and on our capacity to interpret forest responses to environment, should be a major consideration.

Conflict of interest

None declared.

References

Landhausser SM, Chow PS, Dickman LT et al. (2018) Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. Tree Physiol. this issue.


