eXtra Botany
Insight

Tentative Title:
Getting more bark for your buck: Nitrogen economy of deciduous forest trees

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Seasonal nitrogen (N) remobilization is an extensive process in perennial plants requiring communication between N sinks and storage tissues (sources). These plants coordinate changes between expanding buds/shoots and bark to guarantee N storage resources during the dormant season and N utilization during the growth phase. Based on seasonal nitrogen cycling in *Populus*, Li *et al.* (2020) have proposed a novel model using knockdown of bark storage proteins (BSPs) transgenic plants, where auxin production leads to N mobilization from BSPs to expanding buds. This represents a significant step forward in understanding the role of BSP during seasonal plant growth and how N remobilization in trees is regulated.

Little is known about N remobilization in perennial plants. Indeed, trees are challenging models for use in molecular biology research due to their extensive genomes, enormous size, and the difficulty in performing studies as compared to non-woody plants (Kumar *et al.*, 2015; Allona *et al.*, 2019). Although N uptake and assimilation are similar processes in plants, N seasonal cycling is exclusive to perennial trees, which must distribute N from senescing leaves to storage tissues during dormancy and subsequently remobilize the N storage from perennial tissues to growing parts of the plant (Li and Coleman, 2019). This process is important for the N economy and allows trees to reuse N resources rather than external N supplies (Box 1). In *Populus*, during dormancy autumnal leaf senescence initiates protein degradation, and organic and inorganic N compounds are transported within the plant until they reach bark and wood parenchyma cells, where they accumulate mainly in the form of BSPs (Coleman *et al.*, 1993; Cooke and Weih, 2005) or amino acids such as arginine (Arg) (Couturier *et al.*, 2010; Babst and Coleman, 2018). Dormancy is characterized by a short-day (SD) photoperiod, low temperatures, transformation of the apex into a bud, and cessation of leaf primordia growth (Maurya and Bhalerao, 2017). After the dormant phase, BSPs are broken down and N forms are translocated to the expanding buds and shoots, where N is required for growth and development. The dominant amino acid form here is glutamine (Gln), which is an essential building block for protein biosynthesis (Black *et al.*, 2001; Couturier *et al.*, 2010).

Towards a possible model: Regulation of N remobilization by auxin
The plant hormone auxin (IAA), which promotes cell elongation, enhances lateral root initiation and development in growing plants, and is a candidate in mediating N signals from shoots to roots (Kiba *et al.*, 2010). Li *et al.* (2020) provide a comprehensive study on N remobilization regulated by auxin transport. Specifically, they generated six independent lines of BSP RNAi poplar plants and assessed how N availability, bud break and expansion, and switching from LDs (long days) to SDs-LT (short days with low temperature) were affected, as well as DNA microarrays in bark plants comparing LDs and SD-LT. Interestingly, gene

expression in auxin transporters (as well as auxin signaling) and metabolism-related genes increased during LDs associated with BSP catabolism and N remobilization. Moreover, amino acid transporters and proteases were induced during the same treatment conditions (indicating a greater pattern of N storage breakdown). Inhibition of IAA sources in the seedlings (using the auxin efflux inhibitor 1-*N*-naphthylphthalamic acid, NPA) led to dormant conditions, with a general reduction in auxin transporters and protease genes.

BSP biosynthesis and catabolism have long been considered antagonistic processes that define N seasonal cyclings (Box 2; Cooke and Weih, 2005), and represent the first significant step in understanding how trees recycle N and utilize it throughout the year.

Glutamine is the main amino acid transported from the bark to leaves (Couturier *et al.*, 2010). The enzyme glutamine synthetase (GS) synthesizes Gln, and with glutamate synthase (GOGAT) maintains the homeostasis of amino acid biosynthesis. Several previous studies have reported GS family genes in poplar (Castro-Rodríguez *et al.*, 2011; 2015) and have described the important role of GS2 in bark during regrowth (Castro-Rodríguez *et al.*, 2011). Li *et al.* (2020) establish a clear connection and propose an import/export system orchestrated by the glutamine transporters CAT10 and CAT11. Nevertheless, transport regulation in organ-dependent and seasonal conditions remains unclear.

Can N remobilization in trees be made more profitable?

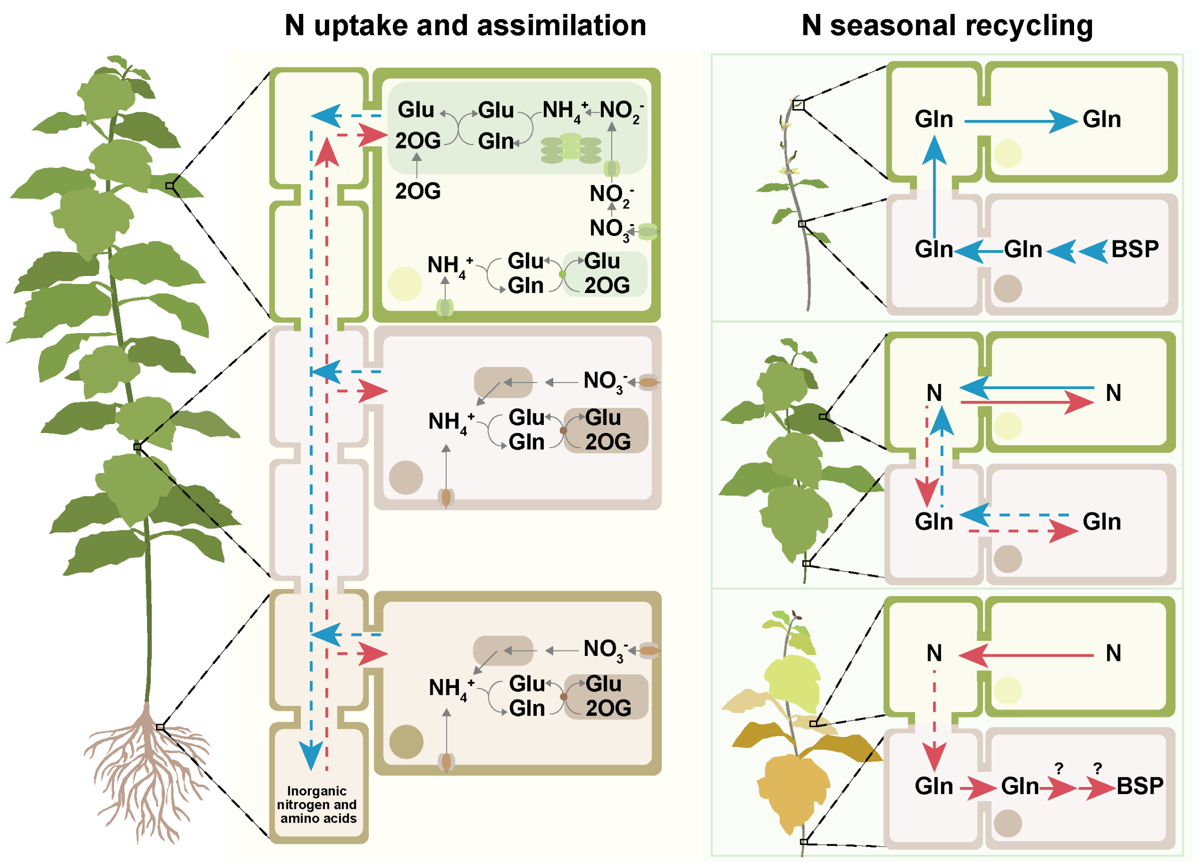
The results provide an excellent basis for identifying the possible actors playing an important role in the N economy of poplar trees. Nevertheless, additional studies have suggested that in addition to cellular and molecular levels of knowledge, different processes could also affect the N seasonal cycling, such as ecophysiological and ecosystem levels (Cooke and Weih, 2005). The present authors have described a model that allows us to identify possible regulatory and transport genes involved in N remobilization. Even if it does not cover a wide spectrum of natural forest populations, the model does provide us with a snapshot of what happens in poplar seedlings under specific conditions. The question is not only how the regulatory switch during N seasonal cycling is turned ON/OFF, but what serves as the switch.

We do know from previous studies that N remobilization brings trees to a unique condition to reuse N resources over the year, and clearly N compounds are on the move between sink and sources tissues within the plant. Understanding the molecular basis of the missing link between the regulation of BSP catabolism and biosynthesis (which occurs mostly in bark), and the framework that coordinates the N transport from sink to sources will allow us to comprehend the ON/OFF part of this mechanism. Babst and Coleman (2018) recently reported a model in which nutrient transport and sensing have important roles in N remobilization, although more studies are needed, particularly regarding the role of key amino acids such as Arg and Gln. Recent studies in conifers (Cañas *et al.*, 2016; Chernobrovkina *et al.*, 2016) suggest that Arg plays an annual dynamic role in N storage and could be affected by temperature, light, and external nutrition supply. Gln has been reported as a signaling molecule in bacteria, yeast, and animals. In plants, Gln is considered to be a signaling molecule due to its role in the regulation of transcription factor gene expression in rice and its interaction with hormones such as cytokinin, which confers a feedback regulation during N uptake and metabolism in poplar (Dluzniewska *et al.*, 2006; Kan *et al.*, 2015). This information leads us to consider that amino acids could act as a sensing system to drive N traffic throughout the different seasons and could therefore operate the cycling switch button in perennial plants.

This begs the question if we can make N remobilization in trees more profitable. Unfortunately, in light of what we know, there does not appear to be any answer in sight. Nevertheless, the new findings of Li *et al.* (2020) are highly meaningful in this context, and novel discoveries resulting from physiological and ecosystem studies will eventually lead us to generate tools to help understand the complex process of N remobilization from genes to ecosystems.

Future trends

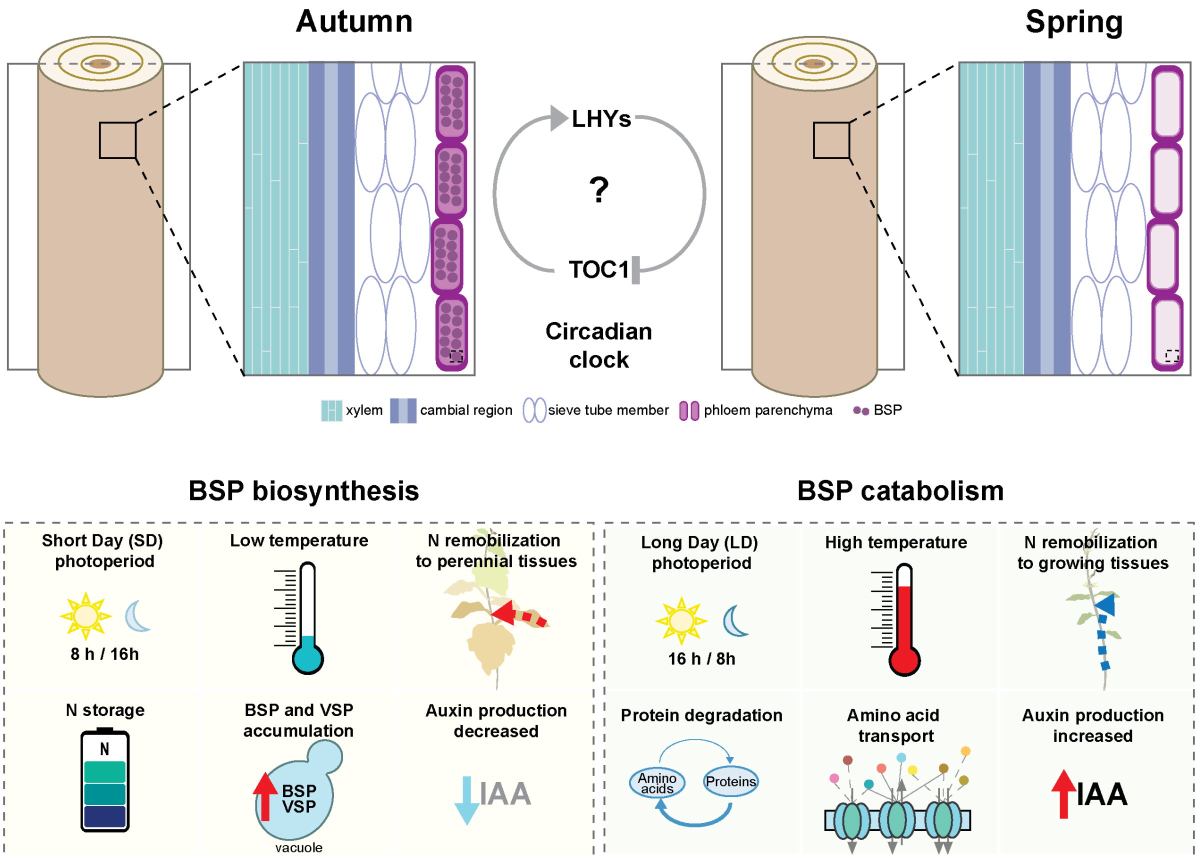
Despite the gaps in our understanding of N remobilization in trees, there is an exciting diversity of research paths in which to focus our efforts. In the future, we recommend using adult trees *in situ* to quantify and describe the experimental model. Since most quantitative studies of N cycling have been addressed (by necessity) in young seedlings and artificial conditions, using adult tress might give us a broader perspective of how N remobilization is managed, and experiments could be designed to include other possible interactions within the tree community. For instance, previous work in trees has successfully shown an enhanced N utilization in transgenic trees (Dubouzet *et al.*, 2013). All of these efforts have brought us new information related to transcriptomics, proteomics, metabolomics, and genomics. These large-scale systems can demonstrate the functionality of many more candidate genes that will be validated for N processes in trees. Examples include using N accumulation to increase the productivity of forest trees and to increase NUE (nitrogen use efficiency) in plants, knowing that N recycling is the main contributing factor to NUE in trees. Li *et al.* (2020) therefore show us that understanding the regulation process of N remobilization is crucial to resolving how plants use N in forest ecosystems.



Box 1: N uptake and assimilation in poplar trees are complex processes that utilize low- and high-affinity transporters to acquire organic and inorganic N forms from the soil. NH_4^+ and NO_3^- transporters are present in the plasma membrane and facilitate allocation inside the cells; once inside, N is assimilated into amino acids. The main step in amino acid biosynthesis is catalyzed by glutamine synthase (GS: EC 6.3.1.2) and glutamate synthase (GOGAT, EC 1.4.7.1, and EC 1.4.1.14) and involves the ATP-dependent condensation of NH_4^+, as well as carbon skeletons in the form of 2-oxoglutarate (2OG) and glutamate (Glu) for the synthesis of glutamine (Gln) (Cánovas *et al.*, 2018). Expression studies (Castro-Rodríguez *et al.*, 2011)

have identified differential roles in two GS isoforms, cytosolic GS1 and chloroplastic GS2. These isoforms are distributed in tissues throughout the plant and contribute to N metabolism in photosynthetic cells, the reassimilation of NH_4^+ released during photorespiration, and N mobilization during seasonal N cycling.

During the new growing season, bark storage proteins (BSPs) are broken down and organic and inorganic N forms are mobilized from storage tissues (sink) to buds and shoots, mainly in form of Gln. N transport is active during the growth phase, followed by N remobilization from senescing leaves (source) to sink, and N is stored as BSPs and/or arginine (Arg) (Babst and Coleman, 2018).



Box 2: Bark storage protein (BSP) is the major vegetative storage protein (VSP) in *Populus* (Coleman *et al.*, 1993). The illustrations on the left represent the radial sections of stems in spring compared to autumn (Cooke and Weih, 2005); the phloem parenchyma cells contain numerous BSPs that disappear during the growth phase in spring and accumulate during dormancy in autumn within perennating tissues. BSP presence is linked by BSP catabolism and biosynthesis; BSP breakdown during spring is characterized by N recycling from sink to source in order to support growth and development, whereas BSP synthesis mobilizes N to storage during dormancy. All of these processes are characterized and regulated by the events listed above. Combining this information, we can visualize the antagonistic role during these different scenarios.

Key words:
Poplar, nitrogen, BSPs, auxin, glutamine, arginine

Journal of Experimental Botany, eraa130 doi: 10.1093/jxb/eraa130

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