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Does the time of the sampling matter in 13C pulse labeling and chasing experiments? A case study on beech seedlings

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13C pulse labeling and chasing is a valuable and very popular tool for determination of the fate and turnover rates of C in plant-soil systems. Continuous isoflux measurements became an accessible reality allowing to cover completely the diurnal variation in label assimilation and respiration fluxes. Label turnover in multiple pools, especially of those located belowground, is more often assessed instead by isolated day-time samplings. By increasing the sampling frequency of belowground compartments we aimed to catch the short-term diurnal variations in label allocation and to link these processes with label dynamics in the aboveground biomass. For these purposes we labeled 3-m height soil-grown European beech seedlings with 13C enriched CO_2 and traced the flow of 13C within belowground plant-soil continuum. Continuous soil isoflux measurements were accompanied by a 3-h-frequency sampling of root and soil material during the first 48 h, followed by a daily sampling in the successive 5 days. The amount of label found in microbial biomass depended partially on the amount of roots in the sample. Microbial biomass C (MBC) and microbial respiration showed very strong correlation, suggesting the possibility to use one as a proxy of the other. MBC enrichment showed a clear diurnal pattern with night-time and early morning peaks. These peaks were similar in shape and shifted by one sampling when compared to root sugars enrichment. Soil respiration showed instead a single bell-shape peak in 13C, likely due to a sequence of peaks of root and microbial origin. 13C flow into soil microbial functional groups was assessed less frequently through phospholipid fatty acid analyses (PLFA). The microorganisms were separated into two distinct groups by the time of the appearance of the label in the single PLFAs. The first group was characterized by a fast appearance of the label and higher enrichment and was composed of Gram negative bacteria and saprotrophic fungi likely living in the rhizosphere and feeding on root exudates, fine root litter and sloughing cells. Night-time assessment and more frequent sampling would be also desirable for this group, showing a similar behavior with MBC 13C. The second group was characterized by delayed label appearance and small enrichment suggesting its presence outside the rhizosphere and secondary 13C utilization. This group was represented mainly by Gram positive biomarkers. We conclude that considering just day sampling may lead to an underestimation of the amount of label allocated to all belowground pools and to a biased estimation of the C allocation velocity in plant-soil system.