FIA-FSP Project Number and Title: Y081136. Potential of stable-isotope-probing of lipids to identify keystone species involved in forest soil nutrient cycling.

Project Purpose and Management Implications:

The project is addressing issues regarding the identification of indicators for soils for sustainable forest management. This project is evaluating the potential of stable-isotope-probing of soil microbial and faunal lipids to understand trophic interactions and identify keystone species involved in forest soil nutrient cycling, which could be used in future as indicators of sustainable forest management. The technique will be applied to soils and fauna from aggregated and dispersed retention treatments at all 3 STEMS replicates to assess the density and arrangement of living trees needed to sustain energy and nutrient fluxes in forest soils.

The findings will make major advances in our understanding of the importance of biodiversity to terrestrial ecosystem functioning and to evaluate the effects of variable retention harvesting on soil diversity and function. The information will enable us to recommend the best design of VR treatments to maintain the soil resource.

The results will be used to revise guidelines for forest management practices to better protect the soil resource and preserve biodiversity. The effect of harvesting on soil organisms and associated functions is a critical knowledge gap that precludes certainty about the sustainability of current and proposed forestry practices. The decisive knowledge achieved through this research will allow BC forest managers to develop and use practices that are based on sound, defensible science and put BC at the forefront of forest research to support sustainable forest practices.

B.C.'s Forest Practices Act, Canada's National Forest Strategy and Canada's Biodiversity Strategy all emphasize the importance of maintaining forest biological diversity, the ecological integrity of forests and ensuring sustainable use of forest resources. Current policies, regulations, and guidelines do not sufficiently consider the importance of soil organisms to the maintenance of ecological functions. This is largely because the scientific information does not yet exist to guide decision-making.

Project start date, length of project and any former numbers or funding sources that apply:

April 2007 3 years.

Methodology overview:

Soils contain an immense population and diversity of fauna and microorganisms and it remains an enigma how such a collection of organisms co-exist and together create the nutritional environment that determines forest composition and function. Although the importance of these organisms in nutrient cycling is well-recognized the structure of the belowground food web and the interactions between these organisms are poorly understood. As a consequence we do not know which of these species are "keystone" and essential for carrying out particular ecosystem processes and what the repercussions of a loss of any of these organisms may have on ecosystem function and resilience. This is largely because until recently we lacked methodologies to study them. Natural abundance stable-isotope analyses ($^{13}C/^{12}C$; $^{15}N/^{14}N$) have recently emerged as powerful techniques

with which to address some of these mysteries and are increasingly being used in studies of food web structure and trophic connections. Most elements of biological interest (e.g. C, H, O, N) have two or more stable isotopes, with the lightest of these present in much greater abundance (e.g. natural abundance of heavy isotopes ^{13}C and ^{15}N is <1%). Natural variations in the ratios of stable isotopes of $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{18}O/^{16}O$ can enable discovery of the origin and flow of these elements in the environment. The technique is based on the fact that consumer's tissues are enriched in ^{15}N by 3.4‰, C by 1‰ relative to its food, in addition many herbivorous food sources have different ^{13}C and ^{15}N content (e.g. leaves, wood, SOM). Therefore, the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios can be used to help identify the consumers' diet and ultimately trace the initial food source. However, the technique has limitations because food sources with similar isotopic ratios cannot be distinguished e.g. different microorganisms. Analysis of biomarker molecules, such as lipids, does not suffer from the same limitations; phospholipid fatty acid (PLFA) and phospholipid ether lipid (PLEL) analysis has been used to assess microbial community structure in soil as different microbial groups possess different signature PLFA (bacteria and fungi) and PLEL (archaea) in their cell membranes. Recently Ruess et al. in Germany analyzed neutral lipid fatty acids (NLFA) together with PLFA to determine the food sources and feeding strategies of collembola in forest soil (PLFA of food sources are incorporated into NLFA without conversion in the consumers). They were able to distinguish between collembolan herbivores, bacterivores and fungivores and predators. A combination of lipid analysis with stable-isotope probing (analysis of the $^{13}C/^{12}C$; $^{15}N/^{14}N$ ratios in the lipids) would offer a tremendous methodological advance with which to study food-web interactions and nutrient fluxes in soil and identify the keystone organisms in terrestrial ecosystems, which has never been done before.

The study sites for this project are the three replicates of the STEMS (Silivicultural Treatments for Ecosystem Management in the Sayward) Experimental Project (EP 1213) (LTRI 026) established by the B.C. Ministry of Forests. The STEMS study is a randomized complete block design with the seven silivicultural treatments having a minimum of three replicates at STEMS 1 and 2. At STEMS 2 there are 4 sizes (5m, 10m, 20, 40m diam.) of the aggregated retention treatment, replicated 4 times. Further details can be found at http://www.for.gov.bc.ca/hre/stems/index.htm.

In year 1 of this project soil faunal and soil samples collected, as part of the Green Tree Retention (GTR) project (Y073049) at the STEMS 2 site in 2005 and 2006 were used to refine the method of lipid extraction and analysis. Mites, collembola and nematodes are the most abundant soil fauna at the site. 10,000 collembola (42 species) and 32,000 mites (92 species) have been collected, identified and stored in ethanol. Similarly, forest floor and mineral soil samples from the site have been stored at -20^{o}C. PLFA, NLFA and PLEL from the soil fauna and soil itself were extracted and identified by gas chromatography mass spectrometry, techniques already established at UBC.

Unfortunately, after extraction and lipid analysis of our stored samples we discovered that the storage procedure for preservation of the collected faunal samples, in ethanol, has caused release of the active lipid fractions and as a consequence we have been unable to use the stored samples for development of this method. As a result we had to collect fresh samples of soil fauna. We took the opportunity to sample the fauna from the third replicate of STEMS, which in November 2007 was just established at Gray Lake, Vancouver Island. We demonstrated on the previous green tree project (Y073049) the

importance of sampling the exact same locations pre- and post- harvest, rather than adjacent uncut areas as controls, because of the spatial variability in these belowground communities. Therefore, the need to sample STEMS 3 was fortuitous and should prove beneficial. We took soil samples for faunal extraction pre-harvest in November 2007 and will return in 2008 to collect post-harvest samples from the site. We have extracted soil mites and collembola from the STEMS 3 samples and have begun lipid extractions from the mites and collembola. Our first task has been to determine the minimum number of individual fauna needed to obtain sufficient lipids for analysis. Our results with these fresh samples look very promising; we are obtaining the spectrum of PLFA we expected and very large concentrations of a limited number of neutral lipid peaks. We have determined that 25 individuals are sufficient to determine feeding strategies. We have now begun analyzing the different mite and collembola species lipids to determine their individual feeding strategies. NLFA, PLEL and PLFA $d^{13}C$ values will be determined using compound-specific isotope-ratio-mass-spectrometry (IRMS) and used to reveal the trophic levels and pathways of the soil food web.

Project scope and regional applicability:

The project is using the STEMS long-term research installations (LTRI026 EP1213) at the Snowden Demonstration Forest, Campbell River (STEMS 1), Elk Bay (STEMS 2) and Gray Lake (STEMS 3) Vancouver Island, as its study sites, in the coast forest district. The findings should be applicable to other forest districts.

Interim conclusions, inference or information that may be useful to forest practitioners and other researchers:

In order to undertake lipid analyses of soil organisms to ascertain belowground food web structure fresh samples of soil fauna are needed, which can be stored, frozen in water for a short time. Lipids should be extracted from defrosted faunal samples rapidly and the lipids can then be frozen prior to analyses. A minimum of 25 individuals of different faunal species are sufficient for the analyses. This study will be the first to attempt such a new approach to understand trophic interactions and food webs in soil using natural faunal samples from the field and that the additional combination of stable isotope analysis on the lipids should be a major breakthrough in understanding C fluxes in these soil food chains.

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