Ecological effects of biochar on stream benthic communities

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Background

Results of field experiments (e.g., Wertman Pond), streamside mesocosm studies and laboratory experiments conducted by DuPont, private subcontractors and University researchers have suggested that biochar is a cost-effective approach for reducing Hg bioavailability. Although numerous studies have been conducted to assess effects of biochar in soil ecosystems, our understanding of potential effects on aquatic ecosystems is greatly limited. Recent experiments conducted by Newman and colleagues (SRST Meeting, October 2013) compared effects of biochar and sedimite on detritus processing by freshwater amphipods. Results showed relatively little effects of biochar, but demonstrated significant reduction of detritus processing in containers containing sedimite. Oleszczuk et al. (2013) observed significant toxicity of several different types of biochar to aquatic organisms (*Daphnia magna*). Toxicity was closely associated with the levels of polycyclic aromatic hydrocarbons (PAHs), natural byproducts of the process used to produce biochar, which varied significantly among biochar types.

In addition to potential toxicological effects of biochar on aquatic organisms, physical effects associated with transport and deposition of these materials are also possible. Loss of interstitial habitat as a result of biochar deposition in stream substrate may influence several different macroinvertebrate groups. Because of variation in particle size of biochar, there is the potential that this material may affect several different groups of benthic macroinvertebrates. For example, filter-feeding organisms such as caddisflies, mayflies and some dipterans will likely be exposed to small biochar particles suspended in the water column. Deposition of small biochar particles to the streambed may affect food resources for grazing insects and collector-gatherers. Before sediment amendments such as biochar can be applied on a larger scale to a natural system such as the South River, it is critical that we understand the potential effects of these materials on stream communities. The community-level mesocosm experiments proposed here will assess potential effects of biochar on each of the major macroinvertebrate functional groups (filter-feeders, collector-gatherers, grazers, predators) present in stream ecosystems.

Objectives and hypotheses

The goal of this proposed research is to examine the potential effects of biochar on the structure and function of aquatic ecosystems. Experiments will be conducted with natural communities of macroinvertebrates exposed to biochar under controlled conditions in stream mesocosms and in the field. Specifically, we will test the hypothesis that exposure to biochar has effects on: survival and community composition of benthic macroinvertebrates, macroinvertebrate drift, immigration rate and community metabolism. Because potential effects are likely to vary with particle size, experiments will also investigate responses of aquatic communities to different size fractions of biochar.

Experimental Approach

The Stream Research Laboratory

Mesocosm experiments will be conducted at the Colorado State University Stream Research Laboratory (SRL), Fort Collins, CO. The SRL consists of 18 stream mesocosms (**Fig. 1**) housed in a greenhouse that receives natural water directly from a deep, mesotrophic reservoir. Water quality in the mesocosms is typical of mountain streams and was characterized by low water hardness (30-38 mg/L $CaCO_3$), alkalinity (25-29 mg/L $CaCO_3$) and dissolved organic carbon (2.5- 3.0 mg/L), cool temperature (12-16.6 ^oC), circumneutral pH (6.7-7.8) and low conductivity (57-89 µmhos). Current in the 20 L microcosms is provided by paddlewheels that maintain a constant current velocity of 0.45 m/s. Each flow-through stream receives water from a headbox at 1.0 L/min, resulting in a turnover time of approximately 20 min. Benthic communities will be collected using a technique that was developed in our laboratory and has been employed for over the past 25 years to assess ecological responses to a variety of anthropogenic stressors (Clements et al. 1988; Kashian et al. 2007; Clements et al. 2013). Briefly, benthic communities will be established on 10 x 10 x 6 cm trays filled with pebble and small cobble substrate placed in the field (**Fig. 1**). Our previous experiments have shown that benthic communities colonizing these trays are very similar to those collected from the natural substrate (Courtney and Clements 2000). After 30 d of colonization, the trays with their associated communities will be removed from the stream, transferred to the SRL and randomly assigned to biochar treatments.

Experimental Design

Mesocosm and field experiments will be conducted to assess the direct and indirect effects of biochar on macroinvertebrate communities. In the field experiment, we quantified macroinvertebrate colonization rates of substrate-filled trays containing biochar placed in small mesh bags. Mesh bags in control trays contained a similar size fraction of gravel. Replicate trays (n = 4) were sampled after 7, 17 and 30 days to test the hypothesis that the presence of biochar would reduce macroinvertebrate colonization.

Different size fractions of biochar may pose different risks to filter feeders, collectors, and grazers, the dominant macroinvertebrates in the South River. In the first mesocosm experiment, benthic communities were exposed to 2 size fractions of biochar during a 10 day period. The experiment consisted of 4 treatments (n = 4): control, large biochar (>1.0 mm), small biochar (<1.0 mm) and large + small biochar. Endpoints (see details below) included macroinvertebrate drift, community metabolism, immigration rate, survival and community composition. Routine physicochemical analyses were conducted throughout the experiment. This experiment is completed and samples are currently being processed. Preliminary analyses of these data indicate a potential effect of the small biochar treatment on community metabolism.

The second microcosm experiment was designed to compare the effects and effectiveness of biochar in the presence of a standard reference toxicant. We choose copper (Cu) for this experiment because it readily binds to biochar and because our previous mesocosm studies allow us to predict direct toxicological effects on benthic communities. This experiment consisted of 4 treatments (n = 4): control, Cu only (50 µg/L), biochar only and Cu + biochar. We predict that the Cu only treatments will reduce abundance of certain metal-sensitive groups (e.g., mayflies), whereas the toxic effects of Cu will be reduced in the Cu + biochar treatments. These experiments are currently underway.

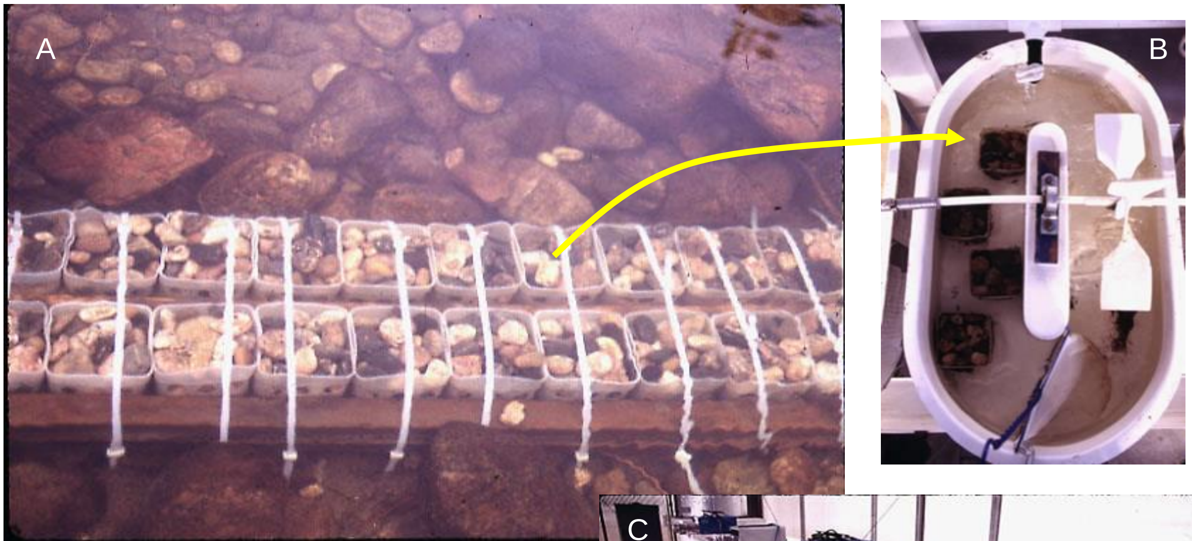

Figure shows colonization trays in the field (A), a top view of a single microcosm with 4 colonized trays (B) and a bank stream microcosms in our greenhouse (C).

Macroinvertebrate drift

We have previously shown that macroinvertebrate drift is highly sensitive to anthropogenic stressors in stream mesocosms (Kiffney et al. 1997; Clements 1999). Macroinvertebrate drift will be was measured by placing a small net immediately downstream from the trays in each experimental stream. Organisms caught in the drift net will be removed from the stream and preserved in 80% ethanol.

Community metabolism

At the end of each mesocosm experiment net community metabolism (gross productivity – total respiration) will be determined using light and dark chambers. Two trays from each mesocosm will be removed and placed individually in either a transparent or an opaque 3.0-L airtight incubation chamber. Each chamber will be filled with mesocosm water and incubated in a water bath to maintain temperature. Community metabolism will be measured as a change in dissolved oxygen concentration in the overlying water in chambers after one-hour incubation.

Immigration rate

It is possible that the presence of biochar in natural substrate will reduce immigration and colonization rates of macroinvertebrates. We will assess the potential avoidance of biochar by measuring macroinvertebrate colonization of natural and biochar substrate in each mesocosm.

Trays (6 cm x 10 cm x 6cm) filled with either natural cobble substrate or substrate mixed with biochar will be placed downstream from the macroinvertebrate communities. At the end of the experiment trays will be removed and organisms will be preserved in 80% ethanol.

Survival and community composition

At the end of the experiment, the 4 trays from each stream mesocosm will be removed and organisms will be preserved in 80% ethanol for determination of effects on survival and community composition. In the laboratory organisms will be counted and identified to the lowest practical level of taxonomic resolution (genus and species for mayflies, stoneflies and caddisflies; tribe for chironomids). We will develop concentration-response relationships between levels of biochar and measures of macroinvertebrate abundance, richness and community composition.

Statistical analyses

All statistical analyses will be conducted using SAS (SAS Institute Inc., Cary, NC, USA). We will use general linear models (PROC GLM) to estimate effects of different size classes of biochar and to establish concentration-response relationships between biochar and benthic community responses (macroinvertebrate drift, community metabolism, immigration, survival). Multivariate analyses (PROC CANDISC) will be used to examine separation and overlap of benthic communities among treatments and to identify sensitive taxa.

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