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Research in Microbiology 163 (2012) 137–141

www.elsevier.com/locate/resmic

Nitrate and ammonium uptake by natural stream sediment microbial communities in response to nutrient enrichment

Nathan D. Bunch, Melody J. Bernot*

Department of Biology, Ball State University, Cooper Life Science Building, CL 121, Muncie, IN 47306, USA

Received 12 September 2011; accepted 10 November 2011
Available online 27 November 2011

Abstract

Anthropogenic activities have increased nitrogen concentration in many ecosystems. Because microbes have higher metabolic rates relative to larger organisms, microbial activity may influence nitrogen movement and degradation significantly in ecosystems. Thus, the ability for microorganisms to adapt to increasing nitrogen concentrations is essential to ecosystem sustainability. We measured sediment microbial community nitrogen assimilation after sustained nitrogen enrichments using in vitro and isotopic techniques. Mixed-microbial communities were exposed to-enriched concentrations of NO_3—N (1 mg l^{-1}) and NH_4—N (30 μg l^{-1}) for four weeks. Each week, filtered water samples were collected from each mesocosm and sediment was removed to quantify rates of nitrogen assimilation by the sediment microbial community. During the fourth week, isotopic tracers $^{15}NO_3$ and $^{15}NH_4$ were added to mesocosms to directly measure nitrogen incorporation into microbial cells as organic ^{15}N. Initial microbial responses to nitrogen enrichment were distinctly different from the sustained microbial community responses. NH_4—N uptake was initially stimulated with NH_4—N enrichments but increased uptake rates were not sustained over time. Sustained responses to changing nitrogen availability equilibrated within 1–3 weeks (depending on nitrogen form), indicating that even though microbial communities can respond to increased availability, potential for increased assimilation is limited.
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Keywords: Nitrogen; Nitrate; Ammonium; Nutrient loading; Uptake; Sediment

1. Introduction

Myriad changes have occurred in ecosystems worldwide due to anthropogenic activities such as urban and agricultural development (Fenn et al., 1998; Galloway, 1998; Howarth et al., 1996; Vitousek et al., 1997). However, it remains unknown as to whether, or more precisely how and when, microbes are responding to these changes. Notably, dissolved inorganic nitrogen concentrations in freshwater has significantly increased throughout North America due to human activity (Middleburg and Nieuwenhuize, 2000; Schlesinger, 1997). Response to this increased nitrogen may include either: 1) inhibition of activity due to toxicity; or, 2) increased or status quo activity associated with adaptation. Both laboratory and field studies have documented varied microbial responses to increasing nitrogen concentrations (Dodds et al., 2002; Hall and Tank, 2003; Hoch and Kirchman, 1995; Kirchman, 1990, 1994) with both toxicity and stimulation of nitrogen assimilation demonstrated in response to increasing nitrogen concentrations. Inconsistencies in results are likely due in part to historical nitrogen conditions for a given ecosystem that may allow for adaptation or tolerance to nitrogen loading in freshwater. To effectively mitigate and manage nitrogen loading to freshwater, a better understanding of adaptive uptake by microbial communities is needed.

Adaptive uptake is defined here as a change in selectivity (i.e., preferential uptake) for nitrogen forms in response to changes in availability over a sustained period of time. Organisms, particularly fast-growing microbial species, may be capable of adapting to different conditions and changing uptake rates over time. Microbes are associated with genetic and edaphic processes, such as genetic alteration, to allow

* Corresponding author.
E-mail address: mjbernot@bsu.edu (M.J. Bernot).

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doi:10.1016/j.resmic.2011.11.004

adaptation to changing conditions (Kearney and Kellogg, 1985). Several studies have identified adaptive nutrient uptake by organisms in natural ecosystems. For example, nutrient enrichment in aquatic ecosystems can change algal community composition (Pedersen and Borum, 1996), likely due to differential affinities for changing nutrient availabilities or species responses that alter competitive interactions. Further, studies have identified inhibition of nitrate (NO_3) uptake by microbes in response to sustained increases in organic nitrogen concentrations (Padgett and Leonard, 1996). Alpine herbaceous species can adapt nitrogen preference in response to sustained changes in availability, likely because these ecosystems are nitrogen-limited (Miller and Bowman, 2003). Microbes can also adapt to xenobiotic compounds resulting in more efficient degradation after sustained exposure (Swindoll et al., 1988). Adaptive uptake is essential if organisms are able to cope with the ever-changing environment. Despite this recognition, understanding of adaptive uptake is limited as most research is conducted at shorter time scales (days) than necessary to quantify sustained shifts in activity. Further, research conducted over longer time periods (months-years) are often field-based and adaptive uptake is difficult to isolate within an ever-changing environment (Bernot and Dodds, 2005).

Using long-term in vitro experiments with the addition of stable isotopes, we quantified nitrogen assimilation rates of natural sediment microbial communities exposed to increased ammonium (NH_4—N) and nitrate (NO_3—N) to assess potential for adaptive uptake. We hypothesized that the microbial community would alter rates of nitrogen assimilation over time to preferentially use the most abundant form of nitrogen. We further hypothesized that initial responses to nitrogen conditions would not be characteristic of the sustained response.

2. Materials and methods

2.1. Culture preparation

One natural sediment microbial community inoculum was used for the mesocosm experiment. This natural community was collected in the spring of 2009 from a stream in central Indiana by randomly gathering the top 5 cm of sediment from several locations and homogenizing the sample through a USGS no. 5 sieve to remove debris and macroinvertebrates. The stream site was characteristic of streams throughout central Indiana with NH_4—N concentrations ranging from 0.03 to 0.05 mg N l^{-1}, NO_3—N concentrations ranging from 0.8 to 4.7 mg N l^{-1}, and DOC concentrations reaching 6.2 mg l^{-1} (Bernot et al., 2006). Sediment was collected <24 h prior to experiment start and preliminary experiments indicated microbial activity did not change after holding periods <72 h.

2.2. Experimental mesocosms

Laboratory mesocosms were constructed and treated with variable nitrogen concentrations as either NH_4—N or NO_3—N. Mesocosms consisted of 2 L homogenized sediment and 3 L stream water in 6 L HDPE plastic containers. Nitrogen treatments were addition of 30 μg l^{-1} of NH_4—N, 1 mg l^{-1} of NO_3—N, or control (no nitrogen addition). Each nitrogen treatment was replicated in four separate mesocosms resulting in 12 laboratory mesocosms. Mesocosms were incubated at room temperature under constant fluorescent light for 4 weeks. Mesocosms were carefully monitored throughout incubation to maintain water volume (appropriately N-enriched stream water was added as necessary).

Each week, initial filtered water samples were collected from each mesocosm and sediment was removed (carefully to ensure minimal disruption) to quantify rates of nitrogen assimilation by the sediment microbial community. Filtered water samples were also collected each week to quantify available nitrogen concentrations. Sediment removed from each mesocosm was placed into 50 ml Falcon tubes (10 ml sediment into each tube, $N = 5$ replicates per mesocosm) with an additional 30 ml stream water from the same mesocosm. Falcon tubes with collected sediment and water were incubated for 3 days followed by nitrogen extraction and collection of filtered water extracts (post samples). Filtered extracts were frozen for subsequent analysis of NH_4—N and NO_3—N concentrations detailed below. Ammonium and NO_3—N uptake rates were calculated each week as the difference between initial and post-incubation water sample concentrations divided by the 3 day incubation time (i.e., mg N d^{-1}). Week 0 uptake measurements were taken immediately after N enrichment.

2.3. Chemical analyses

All filtered water extracts were analyzed for nitrogen concentrations <24 h post-thaw. Ammonium concentration was measured using the colorimetric phenol-hypochlorite technique (Aminot et al., 1997; APHA, 1995) followed by quantifying absorbance for samples and known standards on a Shimadzu dual-beam spectrophotometer. The nitrate concentration was measured by ion chromatography on a Dionex 3000 ion chromatograph also comparing samples to known standards.

2.4. Isotopic analysis

To ensure that in vitro laboratory experiments were robust, direct measurements of nitrogen uptake via ^{15}N incorporation were made in conjunction with sediment assays for nitrogen assimilation. Isotopic tracers ($^{15}NH_4$ and $^{15}NO_3$ as >99% purity $^{15}NH_4Cl$ and $K^{15}NO_3$, respectively) were added to the adaptive uptake mesocosms at the end of 4 week incubation. Isotopes were added to enrich the 15N pool of NH_4—N and NO_3—N ~1000‰. Mesocosms were incubated with the isotope for one week prior to sample analysis. Enrichment of $\delta^{15}N$ to ~1000‰ increased available nitrogen <0.01 mg N l^{-1} in all treatments. Background enrichment of $\delta^{15}N$ in sediment was ~10‰. All isotope samples were prepared in the laboratory by drying mesocosm sediment for 3

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