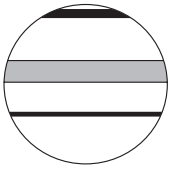

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# Dust deposition drives microbial metabolism in a remote, high-elevation catchment

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**Abstract**

In barren alpine catchments of the Colorado Rocky Mountains, microorganisms are typically carbon (C)-limited, and C-limitation can influence critical heterotrophic processes, such as denitrification. In these remote locations, organic matter deposited during dust intrusion events and other forms of aerosol deposition may be an important C source for heterotrophs; however, little is known regarding the biodegradability of atmospherically deposited organic matter. This study evaluated the extent to which organic matter in Holocene dust and other types of atmospheric deposition in the Colorado Rocky Mountains could support metabolic activity and be biodegraded by alpine bacteria. Microplate bioassays revealed that all atmospheric deposition samples were able to activate microbial metabolism. Decreases in dissolved organic carbon (DOC) concentrations over time in biodegradability incubations reflect the presence of two pools of dissolved organic matter (DOM), a rapidly decaying pool with rate constants in the range of 0.0130–0.039 $d^{-1}$ and a slowly decaying pool with rate constants in the range of 0.0008–0.009 $d^{-1}$. Changes in the fluorescence excitation-emission matrix of solutions evaluated over time indicated a transformation of organic matter by bacteria resulting in a more humic-like fluorescence signature. Fluorescence spectroscopic analyses, therefore, suggest that the degradation of non-fluorescent DOM in glutamate and dust-derived C sources by bacteria results in the production of fluorescent DOM.

**Keywords**

biodegradability, deposition, fluorescence, organic matter

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## Introduction

Remote, alpine environments, located above treeline, are of critical biological importance as well as important sources of fresh water. Barren soils in the alpine environment of the Colorado Rocky Mountains are inherently nutrient poor. Here, microbial communities show limitation for both carbon (C) and phosphorous (P) (King et al., 2008; Lipson et al., 2000). Despite this limitation, an abundance of microbial activity has been found to exist (Brooks et al., 1996; King et al., 2008; Ley et al., 2004; Williams et al., 1997). Many alpine regions are snow covered for more than 9 months of the year, and the short snow-free period provides limited time for the acquisition of C and other nutrients (Ley et al., 2004). Recent studies have suggested that atmospheric deposition may be an important source of C for microbial communities in oligotrophic soils (Mladenov et al., 2012; Oldani et al., 2017). Dust-derived deposition is known to contain non-negligible amounts of C, nitrogen (N), and P (Brahney et al., 2015; Mladenov et al., 2012) as well as minerals (Lawrence et al., 2010; Lequy et al., 2013). Therefore, external inputs of C and nutrients from atmospheric deposition can be particularly important for biogeochemical cycling in alpine catchments (Ballantyne et al., 2011; Mladenov et al., 2011). Indeed, Oldani et al. (2017) found that dry deposition of C represented a substantial C flux (~26.1 kg C $ha^{-1}$ $yr^{-1}$) to an alpine catchment in the Colorado Rocky Mountains. Together, the flux of wet and dry deposition (at 7300 kg C) to the 225 ha Green Lake 4 catchment at Niwot Ridge was comparable to estimated C inputs to the same catchment from soil autotrophic production (at ~10800 kg; Mladenov et al., 2012). Aeolian dust transport has also been shown to be increasing as a result of climate and land use change (Neff et al., 2008), suggesting that the C loading from dust deposition will only increase with time. However, the bioavailability of C in atmospheric deposition and whether it can be used as a C source for alpine microbial communities still remains an unanswered question.

In addition, the role that atmospherically derived N and P play in the biodegradation of C is important. Wet and bulk deposition during Saharan dust intrusion events in alpine catchments has been shown to contain total dissolved phosphorous (TDP) in excess of 10 μg $L^{-1}$ and total dissolved nitrogen (TDN) concentrations often greater than 2 mg $L^{-1}$ (Mladenov et al., 2012). Dust, therefore, may be an important P input for these environments that have been shown to be severely P limited (Elser et al., 2009;

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**Table 1.** Site information and select chemistry for prepared[a] samples.

| Sample name | Sampling date | DOC (mg $L^{-1}$) | TDN (mg $L^{-1}$) | $PO_4$ (mg $L^{-1}$) | $NO_2$ (mg $L^{-1}$) | $SO_4$ (mg $L^{-1}$) | $F^-$ (mg $L^{-1}$) | $Cl^-$ (mg $L^{-1}$) | Ni (ppb) | Cu (ppb) | Zn (ppb) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dusty Snow | 21 June 2010 | 1.5960 | 0.4568 | 0.1853 | 0.0013 | 0.9843 | 0.1026 | 3.6749 | 0.7891 | 2.7969 | 33.3262 |
| Rain | 15 July 2015 | 1.2100 | 0.3913 | 0.0762 | 0.0029 | 0.4942 | 0.0183 | 3.3553 | 0.7693 | 2.2589 | 6.7939 |
| Trough Snow | 12–13 July 2010 | 0.7786 | 0.0517 | 0.0212 | 0.0000 | 0.1739 | 0.0257 | 0.2823 | 1.3554 | 2.6727 | 2.5846 |
| Red Dust | 15–16 February 2006 | 1.1410 | 0.0893 | 0.0469 | 0.0023 | 0.3248 | 0.0261 | 0.3215 | 2.0268 | 3.0767 | 0.9982 |

DOC: dissolved organic carbon; TDN: total dissolved nitrogen.
[a]Concentrations are reported for prepared samples, which consist of dust dissolved in 1 L of DI water concentrated or diluted (depending on initial DOC concentration) to achieve similar DOC concentrations of ~1–1.5 mg $L^{-1}$.

King et al., 2008). Nitrogen, on the contrary, has been shown to have pronounced effects on biogeochemical cycling. Because of atmospheric deposition of N, C-limited soils in the Rocky Mountains are increasingly becoming N saturated (Baron et al., 2009). As a result of N saturation under C-limited conditions, nitrate leaches from soils, as nitrifying processes are allowed to continue unhindered by denitrifying bacteria (Kopáček et al., 2013). Indeed, an increase of nitrate export to surrounding water has been reported in other studies (Baron et al., 2009; Williams et al., 2011). The biodegradability of atmospherically deposited C is relevant in light of the positive feedbacks that microbial heterotrophic activity may have on denitrification and suppression of nitrate leaching into surface waters.

Biodegradability has previously been evaluated using incubation experiments tracking changes in dissolved organic carbon (DOC) concentration, $CO_2$ gas generation, and DOC mineralization (McDowell et al., 2006). The metabolic activity of bacteria has been monitored to evaluate bacterial respiration (e.g. using tetrazolium salts that change color when organic matter (OM) is oxidized (Hice et al., 2018; Roslev and King, 1993)). This rapid colorimetric technique may also serve as an approach to demonstrate that biodegradable organic substrate is available to viable bacteria.

In addition to measurements of C species and metabolic activity during incubations, spectral changes in excitation emission matrix (EEM) fluorescence, namely, in protein-like fluorescence, have been monitored in order to evaluate the biodegradability of dissolved organic matter (DOM) (Fellman et al., 2008). As described by Saadi et al. (2006), fluorescence can behave in two different ways during biodegradation. Fluorescence intensities can decrease because of the actual degradation of fluorescent DOM, or they can increase as non-fluorescent DOM is degraded and transformed into fluorescent DOM. The contribution of protein-like peaks are particularly important as they represent the most labile class of fluorescent DOM. A higher proportion of protein-like fluorescence has been positively linked to DOC biodegradability while greater proportions of humic-like, or more recalcitrant, fluorescence have been negatively linked to biodegradability (Fellman et al., 2008).

To evaluate the biodegradability of atmospherically deposited OM in an alpine catchment in the Colorado Front Range, we used two complimentary experimental approaches in our studies. We added dust collected from wet deposition (rain) and from dust layers in snowpacks, as well as glutamic acid, a readily assimilable C source, to ultrapure water containing *Pseudomonas fluorescens* isolated from the same alpine site. Experiments utilized were as follows: (1) microbial assays with a redox-active probe and (2) biodegradability incubations for which changes in DOC concentrations and three-dimensional fluorescence spectroscopic properties of the DOM were tracked over time. We also performed an additional set of microbial assays and biodegradability incubations for each of the four dust and glutamate solutions with added P to evaluate the influence of P on biodegradability of the external C source.

# Methods

## *Sampling*

A variety of atmospheric deposition samples were collected for this study from the Soddie site (3345 m a.s.l.; 40°03′N, 105°35′W) at Niwot Ridge Long-Term Ecological Research (NWT-LTER) Station in the Colorado Rocky Mountains. A wet deposition sample (referred to as 'Rain') was collected in summer 2015 using an Aerometrics Wet and Dry deposition collection system consisting of 18.7-L bucket containers. The Dusty Snow and Trough Snow samples are snow samples containing dust that were collected on 21 June 2010 and 12–13 July 2010, respectively. Dusty Snow and Trough Snow samples were collected by sampling snow pits at ~10 cm increment depths from the surface. Samples from each depth were pooled to make one large volume composite sample for each site. The Red Dust sample was collected as a large volume snow sample from the snow surface just after a dust event on 15–16 February 2006. For this study, both the Red Dust and Trough Snow samples were freeze dried at the University of Colorado immediately after sample collection. The Dusty Snow sample was kept frozen until thawed for the experiments and analyses of this study. Freeze-dried samples were passed through a 2 mm sieve, and the resulting powders were then added to 1 L of ultrapure (Milli-Q) water. All dissolved samples were then sterile-filtered through 0.2 µm filters (Whatman, nylon) before experimentation and either concentrated via lyophilizer or, in the case of higher concentration samples, diluted to reach a final concentration of approximately 2 mg C $L^{-1}$. Chemical characteristics of all samples are provided in Table 1.

In addition to these field samples, a solution of glutamic acid in ultrapure water was prepared at a concentration of 2 mg C $L^{-1}$ to serve as a readily assimilable C source (glutamic acid) was used. In both microbial assays and biodegradation incubation experiments, an additional sample set of each water type was amended with phosphorous ($PO_4$). Appropriate $PO_4$ concentrations were calculated using the Redfield ratio of C:N:P 106:16:1 (Redfield, 1958). For DOC concentrations of ~2 mg $L^{-1}$, the $PO_4$ addition was determined to be 16 µL of $PO_4$ for every 25 mL of sample.

## *Microbial assays*

Microbial assays were conducted to assess microbial metabolism using 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-Tetrazolium-5-Carboxanilide, also referred to as XTT. XTT is a tetrazolium salt that when introduced to active cells is metabolically reduced to a water soluble, orange colored formazan, for which the absorbance can be measured on a plate reader (Scudiero et al., 1988). XTT absorbance has been correlated with cell growth (Chandran and Das, 2011; Moss et al., 2008), biomass, and oxygen uptake rates (Bensaid et al., 2000) in previous studies, and can be used as an indicator of microbial activity. All samples were inoculated using *P. fluorescens* (treated as above) in duplicate with half of all samples containing 10 µL of Milli-Q water buffer and the other half containing 10 µL $PO_4$ buffer. Members of the *P. fluorescens*

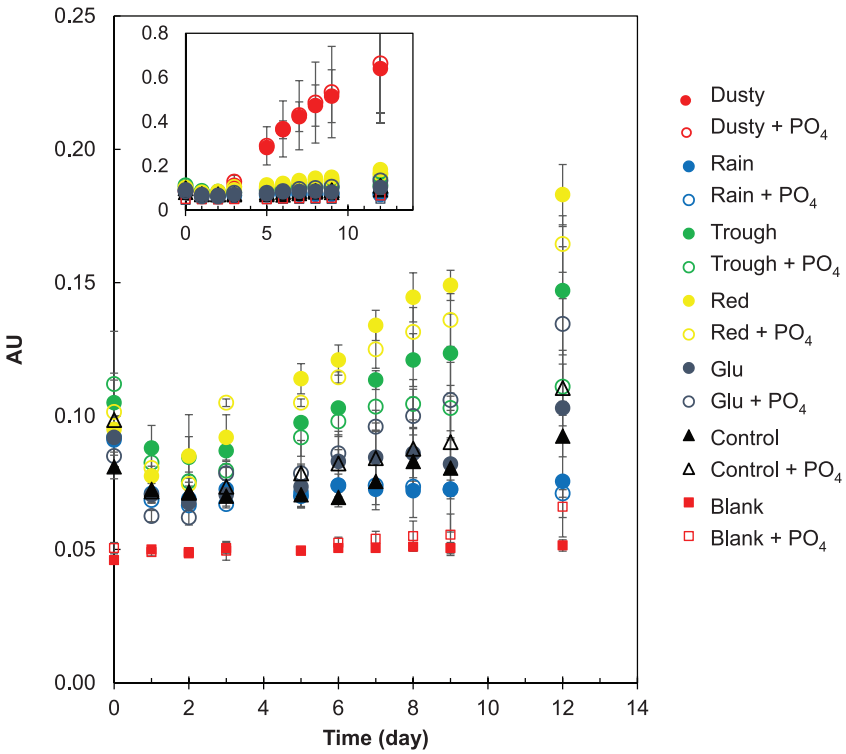


**Figure 1.** Trends in absorbance with time from XTT bioassay samples without $PO_4$ addition (filled circles) and with $PO_4$ addition (empty circles). Error bars represent the standard deviation for the dataset. Inset shows all solutions, including Dusty Snow.

species complex found in the Colorado alpine are metabolically diverse, heterotrophic generalists (Meyer et al., 2004) and will therefore react to a wide variety of organic molecules potentially found in snow, making this species a convenient choice for a bioassay. Color development was measured daily for 12 days using a Molecular Devices Spectramax 190 spectrophotometer at 465 nm.

### *Biodegradation incubation experiments*

Biodegradation incubations using each water type were setup in triplicate bottles for day 0, 5, 12, 21, and 40 sampling. Samples were prepared by filter-sterilizing (using 0.2 µm filters), inoculating with *P. fluorescens* (rinsed two times and resuspended in $MgSO_4$), and incubating in the dark at 25°C. Experiments were also conducted with $PO_4$ addition (as described above) for each water type. On each sampling day, samples were sterile filtered again, and filtrate was collected in two bottles, one that was acidified to pH 2 and one that was kept unacidified. All acidified samples were analyzed for DOC and TDN concentrations with high-temperature combustion on a Shimadzu TOC-$L_{CN}$ Organic Carbon and Total Nitrogen Analyzer using the non-purgeable organic carbon (NPOC) method. The change in DOC concentrations was tracked over time, and a logarithmic curve was fit to the data points to calculate the biodegradability of each sample.

### *Fluorescence*

Fluorescence excitation emission matrices were run on all filtered, unacidified samples used in the biodegradability incubation experiments previously described. Samples were analyzed on a Horiba Aqualog fluorometer at 2 s integration time, excitation wavelengths from 240 to 450 nm at 3 nm increments, and emission wavelengths from 247 to 829 nm at 4.65 nm increments. All EEMs were inner-filter corrected, normalized to the area under the Raman curve (generated from ultrapure water), and blank-subtracted using an ultrapure water blank, with all corrections performed in MATLAB.

Intensities of four fluorescence peaks identified previously (Coble, 1996) were tracked in incubation experiments. These included peaks at excitation/emission wavelengths of 260/380–460 nm (Peak A) and 350/420–480 nm (Peak C), which are associated with humic and fulvic acids, 275/340 nm (Peak T), which is often referred to as the tryptophan-like peak, and 275/310 nm (Peak B), a peak that is similar in position to tyrosine (Coble, 1996).

## Results and discussion

### *Microbial assays*

Microbial assays using the XTT probe showed microbial activity (as an increase in absorbance units) in all but one sample (Rain) (Figure 1). Metabolic activity constants ($k_g$ values) for first-order formazan production ranged from 0.021 to 0.2178 $d^{-1}$ (Table 2) and indicate that most deposition samples were labile to microbial degradation. Although assays containing glutamate did result in microbial respiration with $k_g$ values of 0.021 $d^{-1}$ without $PO_4$ addition and 0.0539 $d^{-1}$ with $PO_4$, assays with atmospherically deposited OM produced higher metabolic activity in *P. fluorescens*.

Dusty Snow exhibited the highest $k_g$ value of all the deposition samples at 0.2178 $d^{-1}$ for the sample alone and 0.2182 $d^{-1}$ with $PO_4$ addition, suggesting that it is the most biodegradable of the dust samples. Dusty Snow also contained the highest $PO_4$ concentration of all dust samples, which can stimulate microbial metabolism in nutrient-poor environments (e.g. Pulido-Villena et al., 2008). It must also be noted that XTT only measures the portion of OM that the bacteria find most bioavailable, not necessarily the entire OM pool. OM with a simpler chemical structure (i.e. substrate that contains basic amino acids), for example, can be a preferred substrate over OM with a more complex structure (Bommer and Ward, 2013). This could be a possible explanation for the poor response of *P. fluorescens* to the Rain substrate. The Rain sample may have contained more complex OM than the other substrates tested. Bommer and Ward (2013), who tested several different substrates, found that those containing β-hydroxyl groups were the least likely to produce formazan while those

**Table 2.** Formazan production, DOC degradation, and increase of Peaks B and T intensity rate constants.

| Sample | Microbial assay | | Biodegradation experiments | | | | Fluorescence | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $k_g$ ($d^{-1}$) | $r^2$ | $k_d$ ($d^{-1}$)[a] | $r^2$ | $k_d$ ($d^{-1}$)[b] | $r^2$ | $k_{peak\ B}$ ($d^{-1}$)[b] | $r^2$ | $k_{peak\ T}$ ($d^{-1}$) | $r^2$ |
| Dusty snow | | | | | | | | | | |
| No addition | 0.2178 | 0.8880 | 0.0390 | 0.4863 | 0.0080 | 0.7830 | 0.0204 | 0.9424 | 0.0138 | 0.7832 |
| $PO_4$ addition | 0.2182 | 0.8966 | 0.0330 | 0.5188 | 0.0090 | 0.8832 | 0.0325 | 0.8477 | 0.0186 | 0.8566 |
| Trough Snow | | | | | | | | | | |
| No addition | 0.0400 | 0.7423 | 0.0130 | 0.0953 | 0.0000 | 0.0909 | 0.0324 | 0.9491 | 0.0286 | 0.9840 |
| $PO_4$ addition | 0.0203 | 0.3007 | 0.0230 | 0.3216 | 0.0060 | 0.8327 | 0.0282 | 0.9562 | 0.0129 | 0.2393 |
| Red Dust | | | | | | | | | | |
| No addition | 0.0697 | 0.9239 | 0.0320 | 0.6096 | 0.0000 | 0.5324 | 0.0278 | 0.9669 | 0.0229 | 0.9184 |
| $PO_4$ addition | 0.0559 | 0.7997 | 0.0250 | 0.5549 | 0.0008 | 0.1154 | 0.0246 | 0.9917 | 0.0221 | 0.9759 |
| Rain | | | | | | | | | | |
| No addition | 0.0000 | 0.0661 | 0.0300 | 0.6096 | 0.0080 | 0.9579 | 0.0414 | 0.9550 | 0.0202 | 0.7528 |
| $PO_4$ addition | 0.0000 | 0.0352 | 0.0320 | 0.5549 | 0.0080 | 0.7174 | 0.0317 | 0.6494 | 0.0127 | 0.1998 |
| Glutamate | | | | | | | | | | |
| No addition | 0.0210 | 0.3628 | 0.4130 | 0.9946 | 0.0010 | 0.0289 | 0.0512 | 0.6690 | 0.0331 | 0.1689 |
| $PO_4$ addition | 0.0539 | 0.7609 | 0.3970 | 0.9935 | 0.0000 | 0.2041 | 0.0320 | 0.2662 | 0.0192 | 0.0349 |

DOC: dissolved organic carbon; OM: organic matter.
[a]$k_d$ values for days 0–5 (rapidly decaying OM pool).
[b]$k_d$ values for days 5–40.

containing aromatic amino acids were the best substrates. Although we were not able to characterize specific compounds in any of the samples, EEMs (Figure S1, available online) show lack of aromatic, amino acid-like fluorescence in the initial (day 0) Rain sample, which only developed after day 12. Further analysis into the chemical structure of individual samples would be needed to better interpret the low bioavailability of the Rain sample.

### *Biodegradability incubation experiments*

Using incubation experiments, we tracked the biodegradability of atmospherically deposited C in an alpine catchment by evaluating the DOC removal over time. Previous studies used bacterial growth measurements to demonstrate that atmospheric deposition is able to support bacterial metabolism in lake waters (Hervàs et al., 2009; Reche et al., 2009). However, the authors concluded that dust provided mainly inorganic substrates for bacterial growth rather than organic C. Pulido-Villena et al. (2008) conducted incubation experiments with two dust types added to filtered seawater and found that dust, as an external source of both soluble reactive phosphorus and DOC, had a fertilizing effect on the nutrient-starved waters of the Mediterranean Sea. Our study adds new knowledge regarding the ability of organic C incorporated in dust to support bacterial growth in alpine soils, which are also C- and P-limited (King et al., 2008). Our results show that DOC removals over the first 5 days of incubation were on the order of 6.5% (for Trough Snow) to 17.1% (for Dusty Snow). The DOC removals we observed for dust samples are only slightly lower than for aquatic organic C sources reported in studies of lower elevation systems conducted over similar incubation times. For example, Fellman et al. (2008) report DOC removals ranging from 20% in a forested wetland to over 40% in fen soil with an incubation time of 30 days. Nevertheless, our study provides evidence that organic C in atmospheric deposition is sufficient to support metabolism of alpine soil bacteria.

Another important observation was that two differently decaying pools of OM appear to be present. Other studies of DOC biodegradability in aquatic systems have also shown that a rapidly decaying pool is degraded followed by a slowly degrading, more recalcitrant pool of OM (Miller et al., 2009; Mladenov et al., 2007). The largest DOC decrease in our experiments occurred within the first 5 days of incubation (Figure 2a). It was not possible to obtain additional time points prior to 5 days. Therefore, for the sake of comparison of the two pools, we fit first-order degradation curves to the data collected from days 0 to 5 to determine the degradation constant for the rapidly decaying pool and from days 5 to 40 to determine the rate of the slowly decaying pool. This exercise may underestimate the rate of the rapidly decaying pool, thereby producing a conservative value that should be revisited in future studies. The glutamate sample exhibited the greatest change with degradation constants ($k_d$ value) of 0.413 and 0.397 $d^{-1}$ for the rapidly decaying OM pool without and with $PO_4$ addition, respectively, corresponding to DOC removals of 87.2 ± 2.2% and 86.1 ± 2.5%. This high degradation rate of this sample was somewhat expected because glutamate (glutamic acid) is a key amino acid in cellular metabolism and therefore readily bioavailable. Moreover, glutamate has been shown to be mineralized by a large percentage of the alpine soil microbial community (Lipson et al., 1999). Nevertheless, glutamate did not degrade as rapidly as some of the atmospherically deposited C in the microbial assays using XTT, which may indicate that dust contains other compounds (lacking in the glutamate addition) that stimulate formazan production.

The Dusty Snow sample, which had the highest conversion of XTT in the microbial assay, also had the highest degradation rates of all the atmospheric deposition samples in the incubation experiments, with $k_d$ values of 0.039 and 0.033 $d^{-1}$ without and with $PO_4$, respectively. The lowest $k_d$ values of 0.013 and 0.023 $d^{-1}$ without and with $PO_4$, respectively, were observed for Trough Snow. Between days 5 and 40, the degradation rates were much slower, ranging from 0.006 $d^{-1}$ for the Trough Snow sample to 0.009 $d^{-1}$ for the Dusty Snow sample.

By contrast, TDN concentrations decreased by approximately 4–45% (Table S5, available online) and did not have two pools degrading at significantly different rates (Figure 2b; Table S6, available online). The $k$ values range from 0.003 $d^{-1}$ (Trough Snow) to 0.006 $d^{-1}$ (Rain) for samples without $PO_4$ and 0.000 $d^{-1}$ (no degradation; Red Dust) to 0.012 $d^{-1}$ (Trough Snow) for samples with $PO_4$ (not shown here, see Table S6, available online). After the initial 5 days, rates were substantially lower ranging from 0.0000 $d^{-1}$ (no degradation) to 0.005 $d^{-1}$ for samples both with and without $PO_4$ addition.

For OM that is deposited in snow, the rapidly available fraction represents an energy source that can be delivered during snowmelt and immediately used by heterotrophic microorganisms in surface

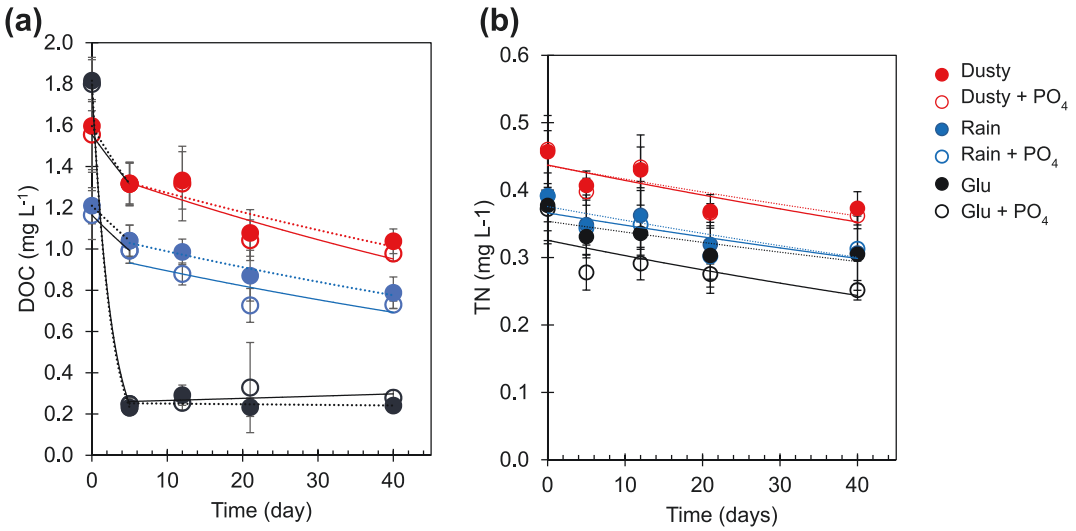


**Figure 2.** Change in (a) DOC concentrations and (b) TDN concentrations of Dusty Snow, Rain, and glutamate samples with and without $PO_4$ addition over 40 days of incubation. Error bars represent the standard deviation of the dataset. Data for other sample types (Trough Snow and Red Dust) shown in Tables S2 and S3, available online.

and subsurface soils. For OM deposited in rain, especially in the summer when DOC concentrations and loadings in rainfall are highest (Mladenov et al., 2012), this pulse of biodegradable OM may represent a hot moment of biogeochemical processing in alpine soils and aquatic environments. The second pool of OM, on the contrary, is more recalcitrant but longer lasting. Our experiments indicate that this pool can be available for microbial communities in soil or aquatic compartments for a month or even months after microbial activity has been initiated. Therefore, atmospherically deposited OM may have a more pronounced effect on biogeochemical cycling in alpine environments than previously expected. In particular, further research is warranted that evaluates the potential for microbial heterotrophic activity, stimulated by atmospherically deposited OM, to support denitrification and suppression of nitrate leaching into surface waters from alpine soils.

### *Fluorescence development during incubation experiments*

Fluorescence matrices from all deposition samples were categorized into regions previously identified as peaks A, M, B, and T (Coble, 1996). EEMs for these samples fell into two groups: one (Dusty Snow and Red Dust) characterized by higher intensities at humic-like peaks A and M, and another (Rain and Trough) with more intense protein-like peaks B and T, which typically reflect microbial contributions to the DOM pool. The EEMs of all samples had a general increase in their respective peak intensities over incubation time, with the greatest intensities occurring at day 40. For example, the Dusty Snow sample (without $PO_4$ addition), exhibiting a more humic-like fluorescence signature, saw an increase in peak M from 0.083 ± 0.002 RU at day 0 to 0.136 ± 0.031 RU at day 40. Similarly, Rain with no $PO_4$, which had a more protein-like signature, saw an increase in protein-like peak B from 0.020 ± 0.005 RU at day 0 to 0.108 ± 0.064 RU at day 40. Dusty Snow EEMs acquired over the course of the biodegradability incubation can be seen in Figure 3 as an example of this trend of fluorescence generation.

Increases in intensity occurred in all samples for peak B over time even if it was not the dominant fluorophore, as in the case of Dusty Snow and Red Dust (see Figure 4). Assuming a first-order increase, peak B fluorescence intensity generation constants ($k_{peak\ B}$) ranged from 0.0204 $d^{-1}$ for Dusty Snow to 0.0512 $d^{-1}$ for glutamate (Table 2). Generation of fluorescence in protein peak regions during biodegradation incubations has also been observed by Saadi et al. (2006), who attributed it to the degradation of non-fluorescent DOM being transformed into fluorescent DOM.

Interestingly, glutamate exhibited a very different pattern in its fluorescence than the deposition samples. Instead of increasing the intensity over time, glutamate initially showed little to no fluorescence at all and peaked the intensity on day 5 for the sample without P and on day 12 for sample with $PO_4$ addition. EEMs did show fluorescence in both peak A and B regions; however, the greatest intensity in glutamate EEMs occurred at an ex/em peak centered at approximately 400/450 nm (see Figure 5).

The overall trend for all peaks to become more intense with time suggests that microbes are transforming non-fluorescent compounds into more fluorescent material than what was originally present in the samples. This means that bacteria contribute to humification of DOM, producing compounds with more double bonds and aromatic C rings that have the ability to fluoresce (Fellman et al., 2010). This is particularly true in the case of glutamate in which no fluorescent material was detected initially, but ultimately the fluorescence intensities increased to >0.08 RU. Although a decrease in protein-like fluorescence (peaks B and T) has typically been attributed to a decrease in biodegradable OM (Baker, 2001; Fellman et al., 2008), the increase in both protein-like and humic-like fluorescence that we observed are more consistent with the type of fluorescence changes that occur during bacterial growth.

### *Effects of P addition on bioavailability and biodegradability*

Concentrations of $PO_4$ in our dust amended solutions that ranged from 0.021 mg P $L^{-1}$ (0.22 µM) in Trough Snow to 0.185 mg P $L^{-1}$ (1.95 µM) in Dusty Snow (see Table 1). Therefore, these prepared solutions were in the same range as or higher than P concentrations recorded in alpine lakes in Colorado (0.15–0.16 µM), Norway, and Spain (0.17–0.34 µM and 0.24–0.25 µM, respectively; Elser et al., 2009) and substantially higher than historical P concentrations found in catchment lakes in neighboring Wyoming (at 0.007–0.012 mg P $L^{-1}$ (0.07–0.13 µM); Brahney et al., 2014).

It was expected that $PO_4$ addition would, to some extent, relieve the P limitation and result in greater bioavailability of OM to the bacteria. Instead, the samples incubated with additional $PO_4$ showed mixed results. Samples used in the XTT bioassay exhibited a higher $k_g$ value in only two of the five samples with $PO_4$ addition (Dusty

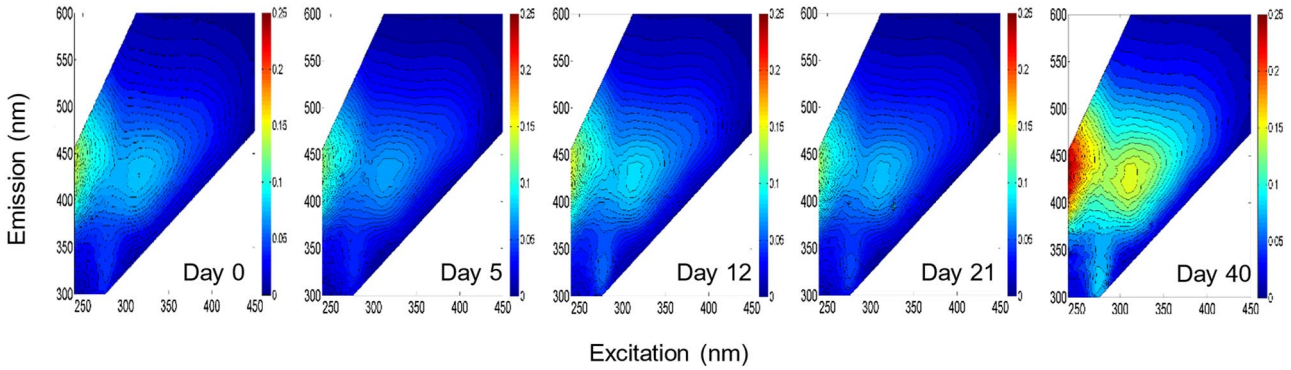


**Figure 3.** EEMs for Dusty Snow samples without $PO_4$ show little change between days 0 and 21, and a substantial increase in all fluorescent peaks by day 40 of incubation.

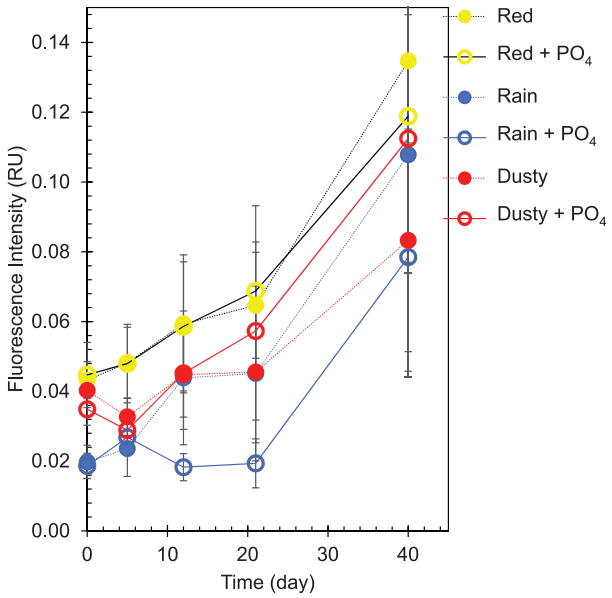


**Figure 4.** Fluorescence intensity of Peak B for Red Dust (yellow circles), Rain (blue circles), and Dusty Snow (red circles) samples both with and without $PO_4$ addition plotted against time. Error bars represent the standard deviations of triplicate experiments.

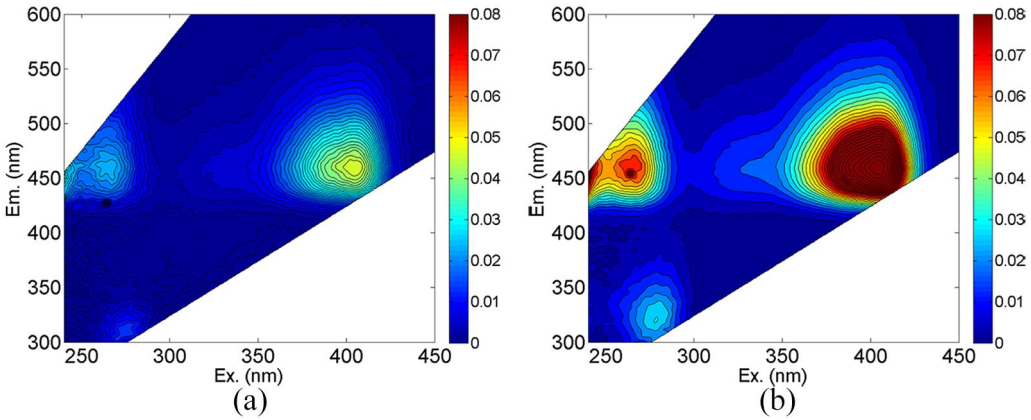


**Figure 5.** EEMs of glutamate solution inoculated with *P. fluorescence* (a) without P addition and (b) with $PO_4$ addition on day 5.

Snow and glutamate), and the other three showed no boost to metabolic activity with $PO_4$ addition. The increase in $k_g$ because of $PO_4$ addition in the Dusty Snow and glutamate samples, however, was not statistically significant in either sample ($p > 0.05$).

Similarly, the addition of $PO_4$ only increased DOC degradation rates in two of the five samples (Rain and Trough Snow) in the first 5 days and three of the five samples (Dusty Snow, Red Dust, and Trough Snow) after day 5. Of those that had increased $k_d$ values, however, none were significantly different ($p > 0.05$) than the $k_d$ values without $PO_4$ addition. This is consistent with the results of the XTT bioassay, in which the effects of $PO_4$ addition did not substantially or significantly increase the bioavailability of DOM to microbes. However, the lack of response to phosphate addition is unexpected, given the known P limitation

of microbes in alpine soils (King et al., 2008). This result may instead suggest that although alpine soils are P limited, atmospheric deposition may already contain P in excess of what is necessary for microbial growth. Indeed, other studies have shown that dust-derived deposition can be a source of P to various environments (Mladenov et al., 2012; Morales-Baquero et al., 2006; Okin et al., 2004). In addition, the deposition sample that degraded the most in this study, Dusty Snow, also exhibited the highest $PO_4$ concentrations, at 0.186 ppm, of all samples measured (see Table 1).

The effect of $PO_4$ addition on fluorescence was also negligible, with $k_{peak\ B}$ and $k_{peak\ T}$ increasing in only the Dusty Snow sample, although not significantly ($p = 0.517$). This result indicates that $PO_4$ has no effect on the rate at which fluorescent material was generated during the incubation experiment. This again would suggest that atmospheric deposition has higher P loadings than the native environment and may not be a limiting nutrient for deposition biodegradation.

## Conclusion

The results of our incubations with diverse atmospheric deposition samples from the Colorado Rocky Mountains demonstrate that the OM contained in atmospheric deposition to Colorado alpine catchments represents a labile substrate for *P. fluorescens*. XTT microbial assays supported these findings with bacterial metabolism becoming activated in all samples except Rain samples. Surprisingly, the addition of $PO_4$ did not significantly influence the biodegradability of any of the samples tested. These results suggest that alpine deposition may already contain enough P to meet the metabolic requirements for biodegradation to occur in alpine catchments.

Based on the biodegradability of C in atmospheric deposition demonstrated here, it may be concluded that atmospheric deposition has an important influence on biogeochemical processes occurring in barren, alpine catchments. In environments that are severely C limited, nitrate reduction has been shown to be greatly decreased leading to nitrate leaching from soils into runoff (Kopaček et al., 2013). The rapidly decaying DOM pool observed in the first 5 days of the incubations may support denitrification as a C source, thereby curbing the effects of N deposition on $NO_3^-$ leaching, particularly during snowmelt when microbes have a limited time frame to access this labile C source. In addition, some atmospheric deposition may already contain a sufficient supply of both labile organic C and inorganic P, which would serve to facilitate or enhance the degradation process.

Fluorescence results for atmospheric deposition samples revealed a transformation of DOM as biodegradation progressed. As the DOM originally present in the sample was degraded, more fluorescent material was produced indicating that microbes were utilizing and transforming the OM. Generation of fluorescent protein-like and humic-like substances, as seen in this study, rather than a decrease in protein-like regions was observed during the biodegradation process and may reflect increased contributions of microbial DOM, such as fluorescent bacterial exudates, to the DOM pool as bacterial metabolism increases.

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### Supplemental material

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