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**Soil nitrogen cycling over two decades following calcium treatment in Hubbard Brook  
Experimental Forest, NH**

**Angelina Uribe**

Master of Environmental Management

Portland State University

June 2020

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## **Abstract**

A watershed-scale calcium addition experiment at Hubbard Brook Experimental Forest, New Hampshire, was performed in 1999 to better understand how forest ecosystem function and biogeochemistry are affected by changes in pH. Significant increases in forest growth and acid neutralizing capacity of the soil and stream water were reported in the six years following the calcium addition, but the expected stimulation of the nitrogen cycle was not observed. The aims of this study were to 1) examine the 20-year dataset of soil nitrogen dynamics following the experimental calcium addition in the treated watershed and paired reference area to understand longer term trends and effects, and 2) explore connections between soil nitrogen processes and watershed nitrogen export in stream water observed in the calcium-treated watershed since 2013. Since 2002, soil pH was higher in the calcium-treated watershed than the reference, with stronger differences seen in surface horizons, but declined over time and converged toward the reference conditions by 2018, throughout the soil profile. Soil nitrogen cycling measurements were not consistent with the changes in soil pH, and temporal trends were different depending on the variable in question. Differences in temporal trends in nitrogen cycling variables were often more pronounced as a function of soil horizon than Ca treatment. However, interesting trends were seen in certain variables, with effects of Ca treatment diverging from long term trends in the reference area for some variables while converging toward reference conditions for others. There was no strong evidence from microbial nitrogen transformations at the plot scale to explain the nitrate export from the calcium treated watershed observed by other researchers (Rosi-Marshall et al. 2016). Because pathways in soil nitrogen cycling did not show consistent trajectories over time in response to the calcium treatment, understanding how changes in pH and other environmental variables, along with competition for nitrogen between plants and soil microbes, differentially affect these various processes remains a challenge in forest ecosystem science.

## *Introduction*

As a result of fossil fuel combustion, the United States experienced decades of increasing emissions of nitrogen oxides and sulfur oxides, which combine with water in the atmosphere to form nitric and sulfuric acids, resulting in acid deposition. While the 1990 amendment to the Clean Air Act (1970) helped to curb emissions of nitrogen oxides and sulfur oxides from industrial sources (Likens 2004), acid deposition had long term detrimental effects on soils, streams, and forest growth long after deposition was slowed (Tomlinson 2003; Likens 2013; Rosi-Marshall et al. 2016). Acid deposition on forest soils leads increases the concentration of hydrogen ions ( $H^+$ ), stripping cations such as magnesium, manganese and calcium (Ca) from soil minerals and leading to depletion of these important plant nutrients.

Northern hardwood forests have been particularly exposed to these effects, due to high levels of acid deposition in the northeast US from emissions by coal-burning power plants in the Midwest and to their occurrence on shallow soils with base-poor parent materials. In the Hubbard Brook Experimental Forest (HBEF), New Hampshire, forest growth has been significantly affected by Ca depletion as a result of acid deposition, with declines in sugar maple and spruce stands and overall forest biomass accumulation (Bailey et al. 1996; Likens et al. 1996; Groffman et al 2006).

In an effort to understand the effects of acid deposition on soil calcium depletion and forest growth, researchers at HBEF experimentally treated a whole 11.8 ha watershed (WS1) by adding 850 kg Ca ha<sup>-1</sup> of pelletized calcium in the form of wollastonite (CaSiO<sub>3</sub>) in 1999, to replace Ca lost over 60 years of acid deposition (Groffman et al 2006). Researchers predicted that Ca additions would increase the soil base saturation, pH, and acid neutralizing capacity (ANC) of soil solution and stream water, resulting in more rapid nitrogen (N) cycling and a sustained increase in forest growth (Horsley 2000; Groffman 2006; Rosi-Marshall et al. 2016).

Ecosystem effects of the experiment were monitored intensively starting in 1998, a year before the Ca addition, in the treated (WS1) and the reference area (west of WS6 or “Bear Brook”; Groffman et al. 2006). Calcium-mediated increases in soil pH from 3.5 to 4.5 were observed, and forest growth was stimulated with Ca addition, (Johnson et al. 2014; Battles et al. 2014), leading to predictions of a “tightening N cycle,” in which N-limited trees accumulate N efficiently in biomass, decreasing watershed N export (Groffman and Fisk 2011).

This hypothesis appeared to be supported by streamwater chemistry monitoring, until a marked increase was observed in stream dissolved inorganic nitrogen export from the Ca-treated watershed in 2013 (Rosi-Marshall et al. 2016). Despite declines in atmospheric N deposition regionally, the Ca-treated watershed in more recent years became a net source of inorganic N, with streamwater N exports exceeding N inputs in precipitation (Likens 2013, Rosi-Marshall et al. 2016).

This evidence that Ca enrichment could turn a watershed from a sink to a source of N suggested multiple potential mechanisms. Given what is known about N cycling and pH, the added Ca should increase soil pH, increasing rates of soil microbial N cycling as follows. Decomposition of soil organic matter would be stimulated, which can increase gross rates of N mineralization and immobilization (Hart et al. 1994; Groffman et al. 2006). Net N mineralization may be reduced, however, when immobilization is stimulated more than mineralization (Groffman et al. 2006). Nitrification and denitrification have also been shown to be sensitive to soil pH, and would be expected to increase with less acidic conditions and higher supplies of N and dissolved organic carbon (C) (Johnson et al. 2014; Groffman et al 2006).

In the first five years following Ca additions, soil microbial biomass C and N declined, but there was not a strong response in soil microbial N cycling rates, contrary to the predictions in the original experiment (Groffman et al 2006). There were declines in the N content of the microbial biomass, potential net and gross N mineralization rates, and soil inorganic N pools in the Oi/Oe horizon of the treated watershed (Groffman et al. 2006). Yet in the longer term, watershed-scale analysis, mass-balance estimates of soil C and N pools and stream nitrate losses suggested that the Ca addition accelerated decomposition, N mineralization, and increased N supply rates (Rosi-Marshall et al. 2016). In addition, recent evidence supports that Ca availability in these organic soils influences mineralization of soil organic C and N primarily by stabilization processes and secondarily through pH effects on organic matter solubility (Minick et al. 2017).

These contrasting findings between earlier and more recent studies following the Ca addition at different spatial scales raise interesting questions about longer term effects on soil C and N cycling rates under elevated pH, as well as the effects of long-term trends in environmental factors, such as precipitation and soil moisture, that could be affecting both watersheds (Groffman et al. 2012). In order to address these apparent inconsistencies, it is necessary to take a closer look at the long-term record soil C and N cycling measurements since Groffman et al (2006). Through the Hubbard Brook Long Term Ecological Research Network (<http://www.hubbardbrook.org>), a 20-year data set consisting of ongoing measurements of soil C and N dynamics in the Ca-treated watershed and reference area was available for analysis. The aim of this study was three-fold: (1) to learn, build and operate Nitrogen-Free Atmosphere Recirculation Method (NFARM), (2) to assist with 2018 soil collection and N cycling and NFARM and (3) to use the long-term data set to answer 4 major questions:

- (a) How has soil pH changed over time in the treated watershed (WS1) 20 years after the Ca addition?
- (b) How does elevated soil pH affect nitrogen cycling rates over time?
- (c) Was there an increase in soil N mineralization and nitrification rates, and/or a decrease in soil denitrification rates, that could explain the increase in stream nitrate export observed in 2013?
- (d) How does soil depth affect N cycling trends, and where in the soil profile are the Ca-treatment effects most pronounced?

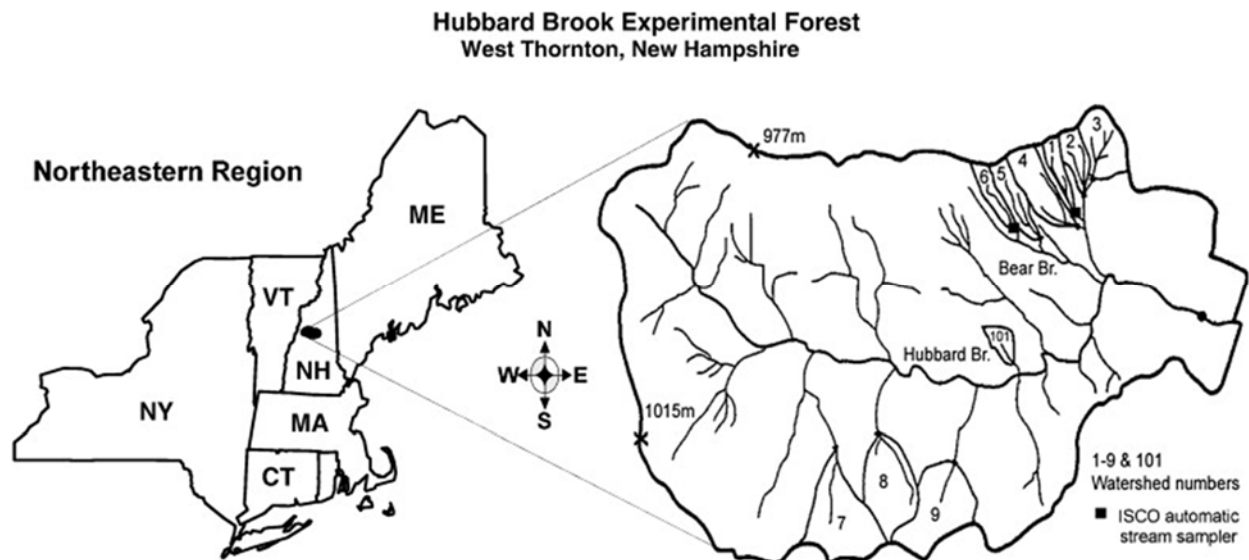
## Methods

### *Site Description*

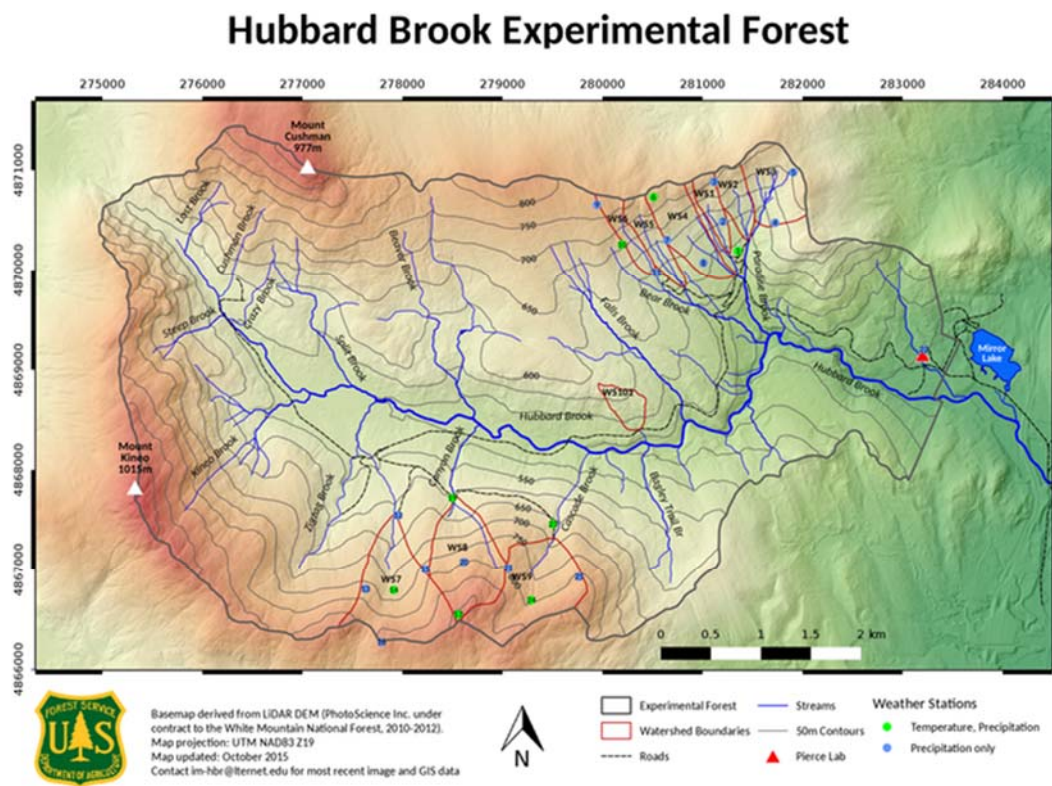
Located in the White Mountain National Forest in New Hampshire, USA (43°56'N, 71°45'W; <http://www.hubbardbrook.org>), HBEF is a typical northern hardwood forest. Soils are Spodosols (Typic Haplorthods), developed on base-poor unsorted basal tills, resulting in shallow and acidic soils that sensitive to increased acidity from acid deposition. The vegetation is characterized by American beech (*Fagus grandiflora*), sugar maple (*Acer saccharum*), and yellow birch (*Betula alleghaniensis*). Streamflow and stream chemistry in the experimental watersheds in this study (WS1; treated, west of WS6; reference) have been monitored continuously since 1963.

Soil samples were collected from plots at four elevations in the treatment and reference areas at HBEF (Fig. 1 & 2) as described by Fiorentino and others (2003). In both watersheds, five replicate plots were established in the four elevation zones corresponding to low hardwood (520–560 m), mid-hardwood (600–650 m), high hardwood (725–750 m), and spruce/fir/white birch (770–850 m) vegetation zones (Groffman 2006). Both watersheds are located on south

facing slopes (20–30% slope) and had similar vegetation along an elevational gradient from 450 to 800 m above sea level.



**Figure 1.** Regional map showing location of Hubbard Brook Experimental Forest.



**Figure 2.** Hubbard Brook Experimental Forest and experimental watershed locations.

### *Soil Sampling for Biogeochemical Analysis*

A 5 cm diameter PVC pipe, about 15-20 cm long, split lengthwise and taped together, was collected to take all samples to a depth of 10-15cm. The corer was removed from the soil and opened with the intact soil core inside. The sample was split into three subsamples (Oi/Oe, Oa/A, and mineral horizons) in the field, and the depth represented by each horizon was measured before placing the subsample into a plastic bag. At each plot, two to eight soil samples were collected from each of the 40 sampling plots (4 elevations  $\times$  5 replicate plots  $\times$  2 watersheds) and were composited by horizon (Oie, Oa, and the top 10 cm of the mineral soil) at each sample date. Samples were consistently collected every year in July, and occasionally also in May and October. To eliminate potential biases due to samples not collected in spring and fall in many years, this analysis focuses on the July samples only.

Samples were kept on ice and transported to the laboratory (Cary Institute of Ecosystem Studies, Millbrook, NY) where they were stored at 4° C between sampling and analysis (from less than 1 week to up to three weeks). Samples were homogenized by hand: all large rocks, roots, and other non-decomposed organic material were removed, and samples were thoroughly mixed. All samples were held at field moisture before analysis. Variables measured were: gravimetric water content, potential net N mineralization, potential net nitrification, denitrification potential (as denitrification enzyme activity), 2M KCl-extractable nitrate and ammonium concentrations, and soil microbial C and N content, as described in detail in Groffman et al (2006). These measurements continued from 1998 to 2018 (traveled to HBEF to assist with the collection and analysis of 2018 soils).

### *Soil Sampling for NFARM analysis:*

Two replicate intact cores were taken via split core method from two sites at each elevation (32 total) with 2 cm diameter corers and will remain unhomogenized. Each core was cut to approximately 10 cm and placed in a bag (rolled and taped to maintain core shape). Samples were kept on ice and transported to Cary Institute of Ecosystem Studies in Millbrook, NY. One core from each site was homogenized by hand as described above. This homogenized core was then used for gravimetric water content analysis and KCl extraction for inorganic N content. Samples were stored at 4° C between sampling and analysis (from less than 1 week to up to three weeks). All samples were held at field moisture before analysis (Groffman; hubbardbrook.org).

The second core remained intact (unhomogenized) and was used for NFARM analysis. NFARM analysis was performed at Cary Institute of Ecosystem Studies in Millbrook, NY in July of 2018 (construction of the NFARM at Portland State University was completed in Summer 2019). The NFARM, which is a modified Shimadzu GC-2014, allowed us isolate the denitrification process from background atmosphere in intact cores incubated in gas-tight jars under optimal He-O<sub>2</sub> mixture conditions. Background atmospheric N<sub>2</sub> was removed in order to capture and isolate soil N<sub>2</sub> production (Burgin et al. 2010, Wang et al. 2011). For the purposes of these incubations, a 20% O<sub>2</sub> – 80% He mixture was used.

### *Data Analysis Methods*

The long-term data set for both reference and treated watersheds excluded samples collected outside of the summer season. Mean values of variables in Table 1 were calculated over all plots and elevations for each watershed by year and soil horizon. Results were represented by time series, with standard error and linear trendlines shown to illustrate spatial

variability and temporal patterns, respectively. NFARM data will be analyzed for future publication.

**Table 1.** Nitrogen cycling variables measured.

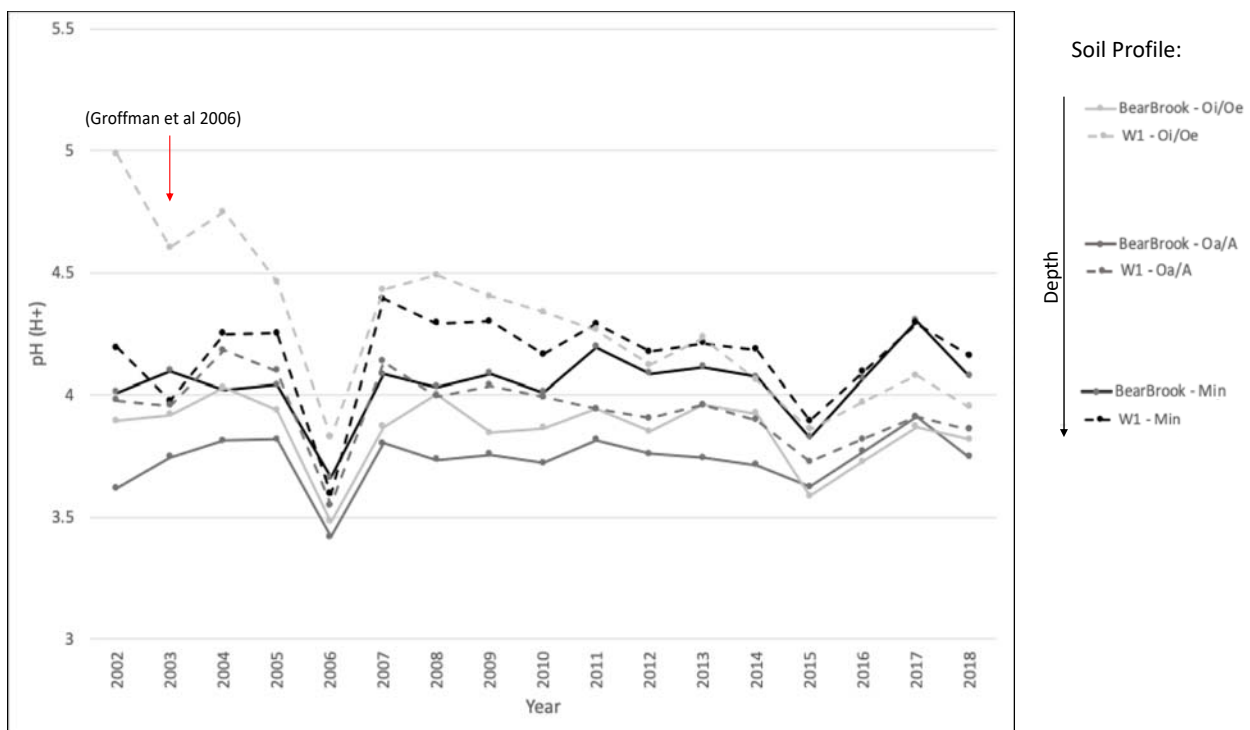
BIOC	Microbial Biomass Carbon
RESPC	Respiration Carbon
BION	Microbial Biomass Nitrogen
NO3	Nitrate
NH4	Ammonium
NIT	Nitrogen Content
MIN	Nitrogen Mineralization
DEA	Denitrification Potential
H2O	Water Content



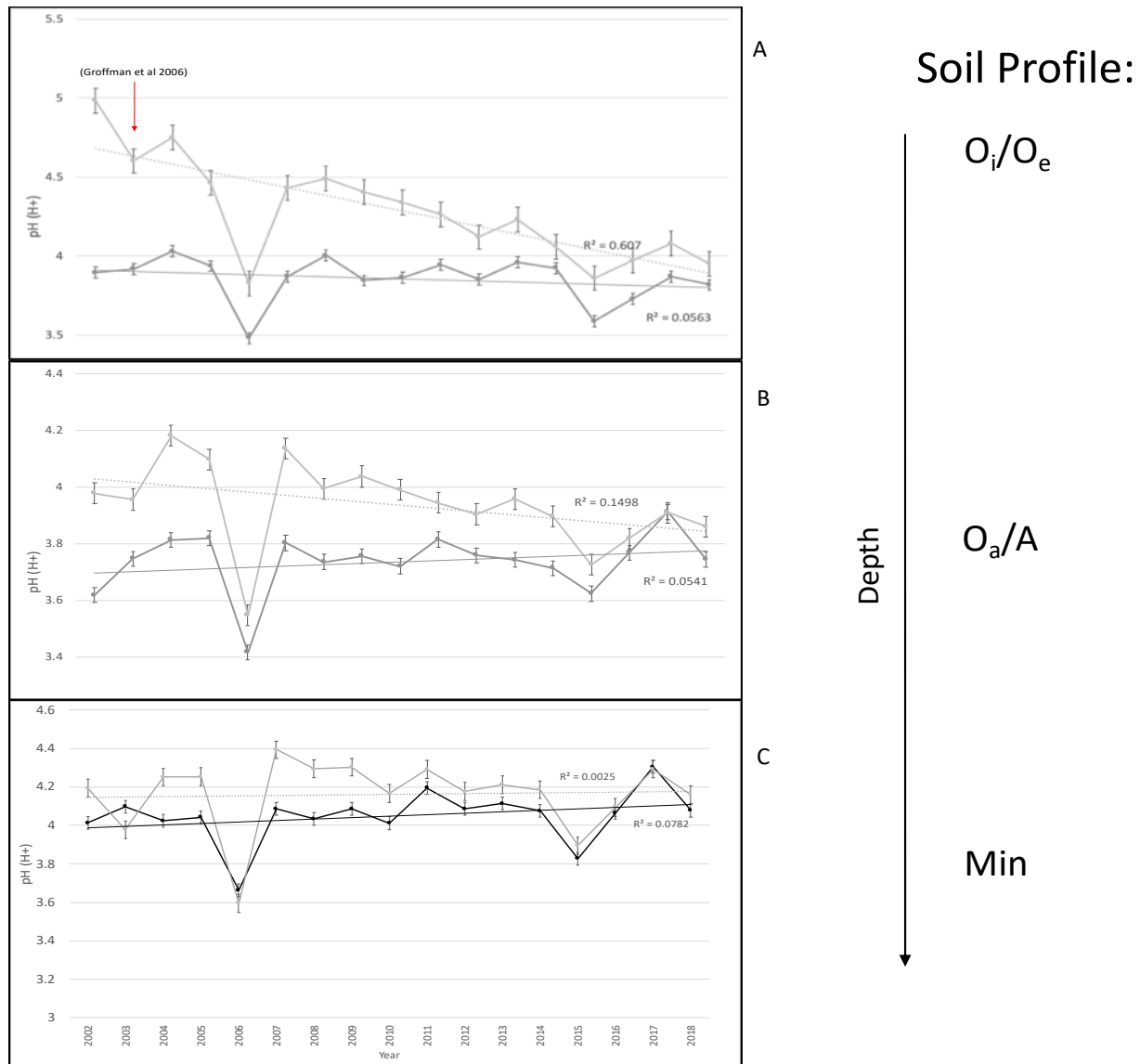
## Results

### Soil pH

Calcium addition initially increased soil pH compared to reference area, but the effects of the treatment decreased over the 20-year period, showing a convergence of soil pH (Fig. 3). Differences between the treated watershed and the reference area were most pronounced in the surface soils (Oi/Oe horizon), while effects of the treatment were more muted with increasing depth in Oa/A and mineral horizons (Fig. 4). As the Ca pellets were applied at the surface, the initial greater effects of treatment on the two horizons closer to the surface are not surprising; differences in soil pH in the mineral horizon emerged later (in 2004; Fig. 4). The greater variability in soil pH over time in surface soils in both watersheds was also expected, due to greater biological activity, exposure to environmental conditions, and inputs of organic matter at the surface.



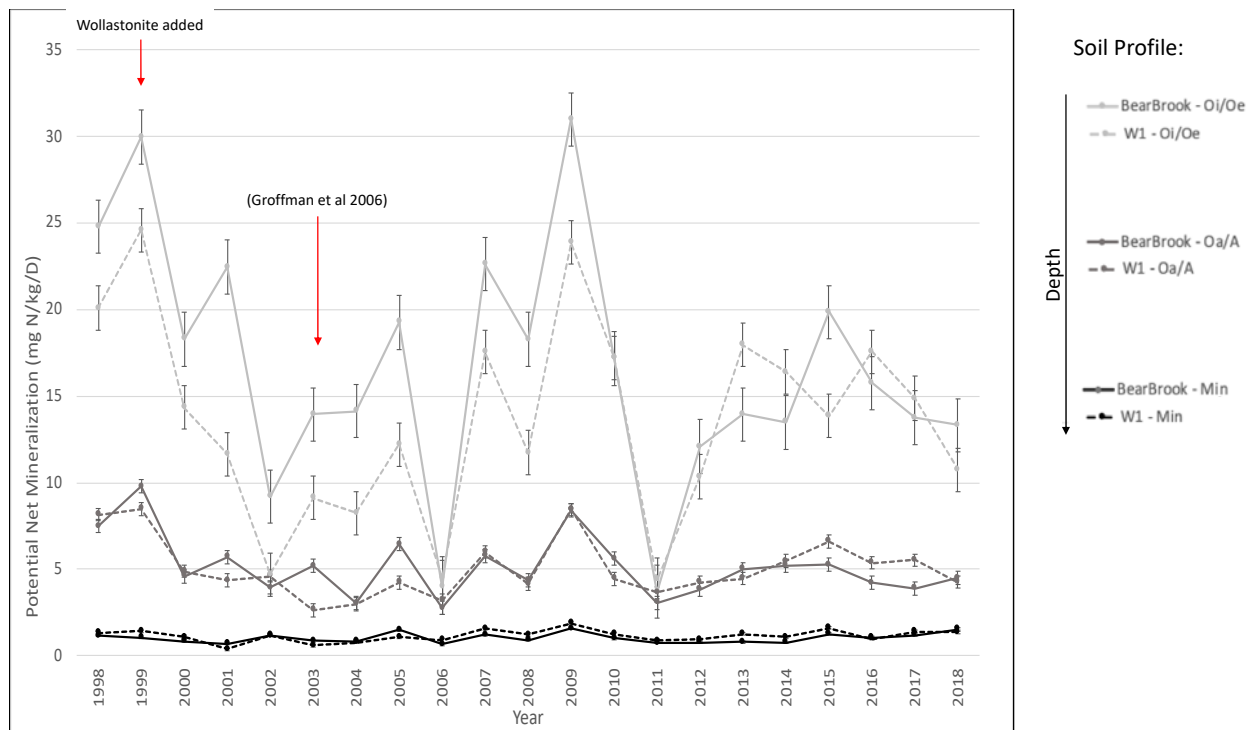
**Figure 3.** Annual average pH by soil horizon over 20-year period in the Ca-treated (WS1; dashed lines) and reference (West of WS6; Bear Brook; solid lines) watersheds. 2016 data was extrapolated by finding the midpoint between the 2015 and the 2017 averages. Arrow indicates the dataset in the first analysis published in 2006.



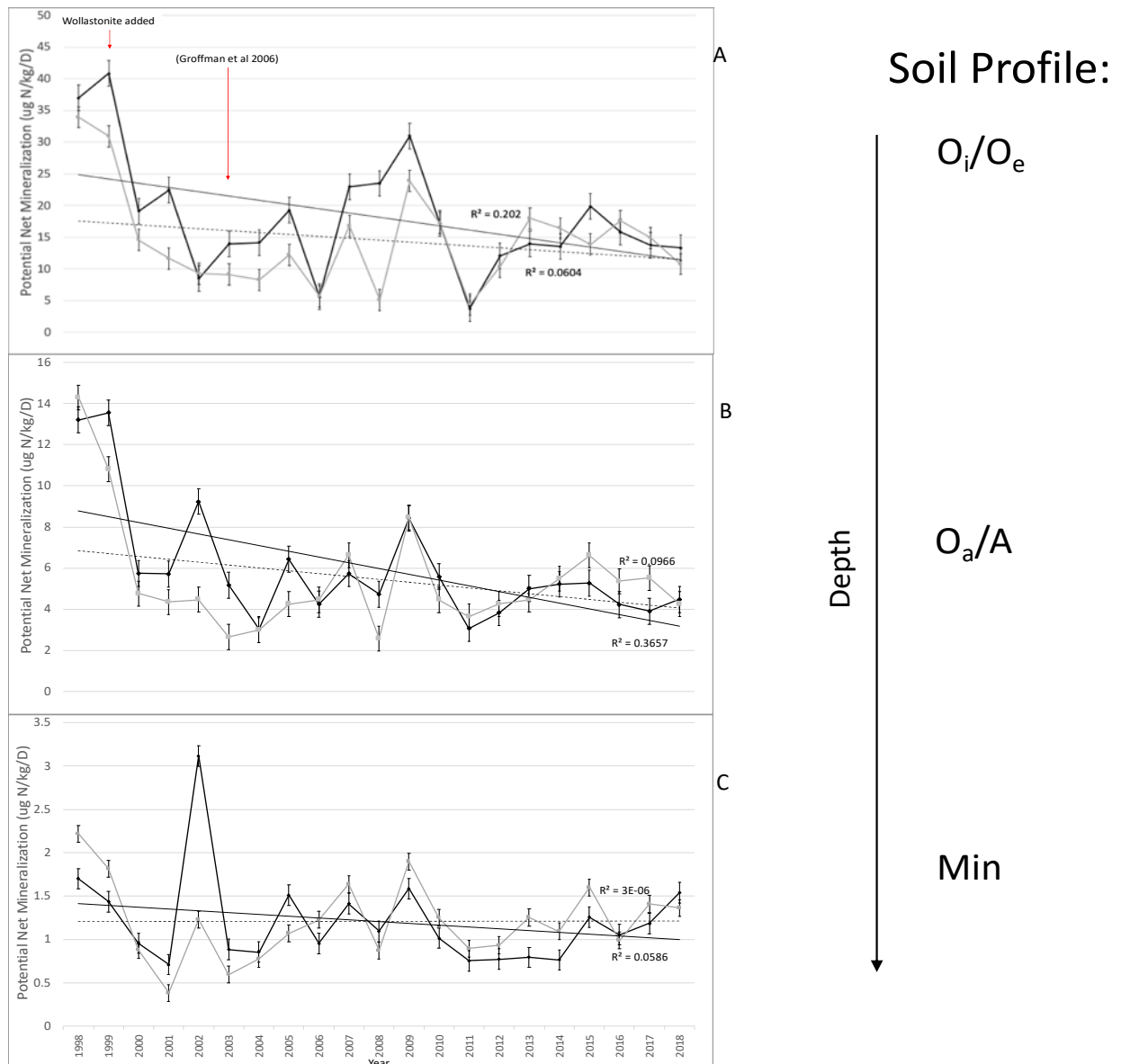
**Figure 4.** Annual average of soil pH in the three measured soil horizons:(A:  $O_i/O_e$ , B:  $O_a/A$ ; C Mineral) in the Ca-treated (WS1; black lines) and reference (West of WS6; Bear Brook; grey lines).

### *N Mineralization Rates*

Potential net N mineralization rates were lower in the Ca treated watershed initially, with differences disappearing by 2010. Overall there was a negative trend in N mineralization over time in both watersheds, but steeper declines were found in the reference area (Fig 6). Surface soils ( $O_i/O_e$ ) and  $O_a/A$  horizons had higher N mineralization rates, steeper declines over time, and greater temporal variability than mineral soils, in which there was no linear trend in either watershed (Fig 6).



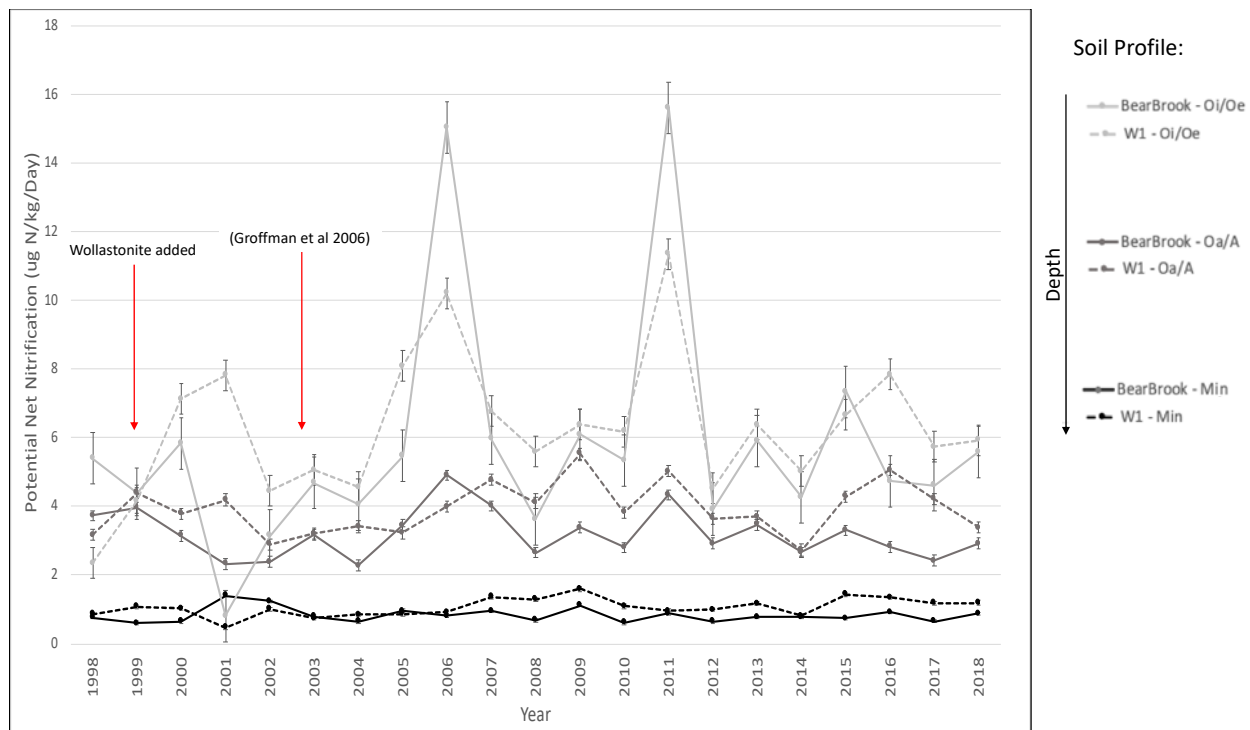
**Figure 5.** Annual average potential net N mineralization over 20 year period in the Ca-treated (WS1; dashed lines) and reference (West of WS6; Bear Brook; solid lines) watersheds. Arrows indicates the date of Ca treatment and the dataset in the first analysis published in 2006.



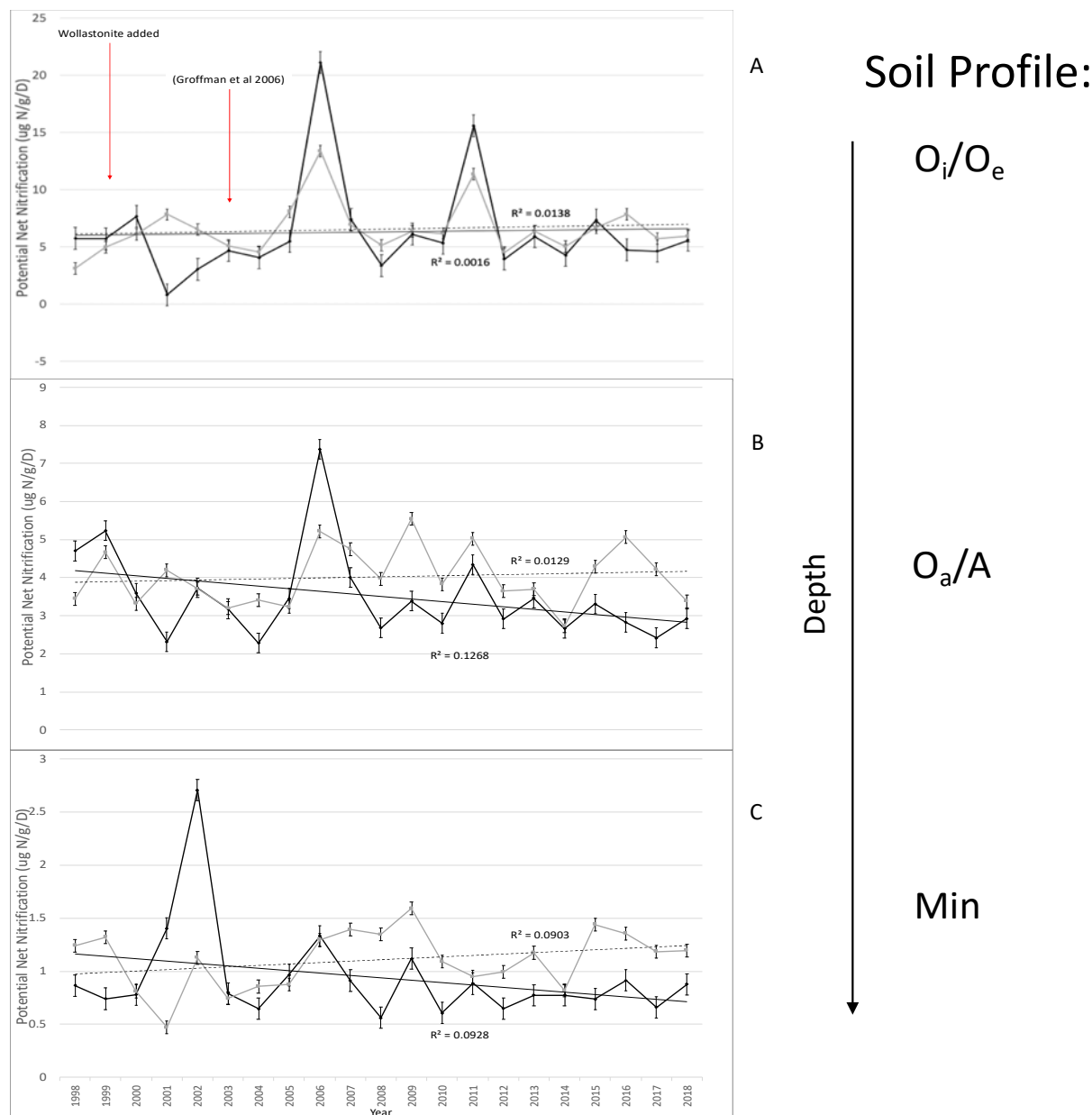
**Figure 6.** Annual average of potential net N mineralization rates in the three measured soil horizons (A: Oi/Oe, B: Oa/A; C Mineral) in the Ca-treated (WS1; black lines) and reference (West of WS6; Bear Brook; grey lines).

### Nitrification Rates

Nitrification rates were consistently higher in the Oi/Oe horizons, followed by Oa/A, and mineral soils in both watersheds (Fig 7). Interestingly, the pattern of temporal trends as a function of soil horizon was opposite that seen in N mineralization: there was little difference between watersheds and no trend over time in surface soils, whereas declines in N mineralization rates over time became steeper with increasing depth in the reference plots (Fig 8). In the Oa/A and mineral horizon, there was a positive trend in N nitrification rates over time, and consistently higher nitrification rates after about 2006 in the Ca treated watershed (Fig 8).



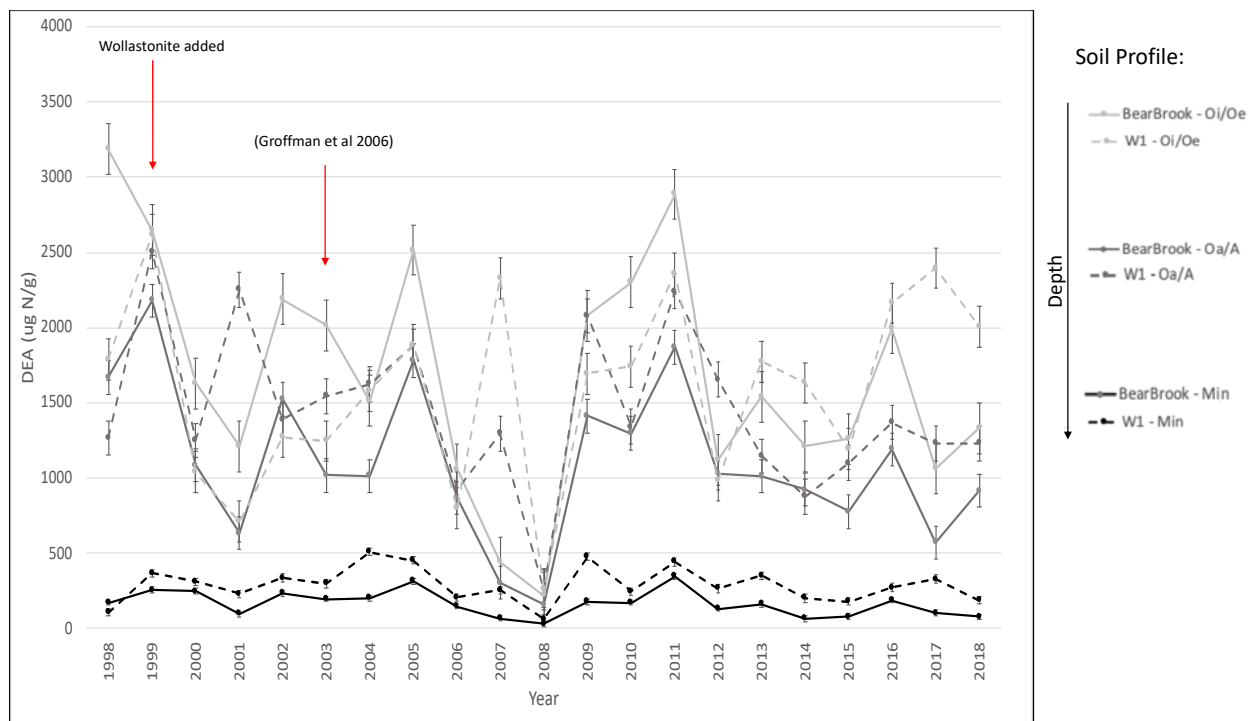
**Figure 7.** Annual average potential net nitrification over 20 year period in the Ca-treated (WS1; dashed lines) and reference (West of WS6; Bear Brook; solid lines) watersheds. Arrows indicates the date of Ca treatment and the dataset in the first analysis published in 2006.



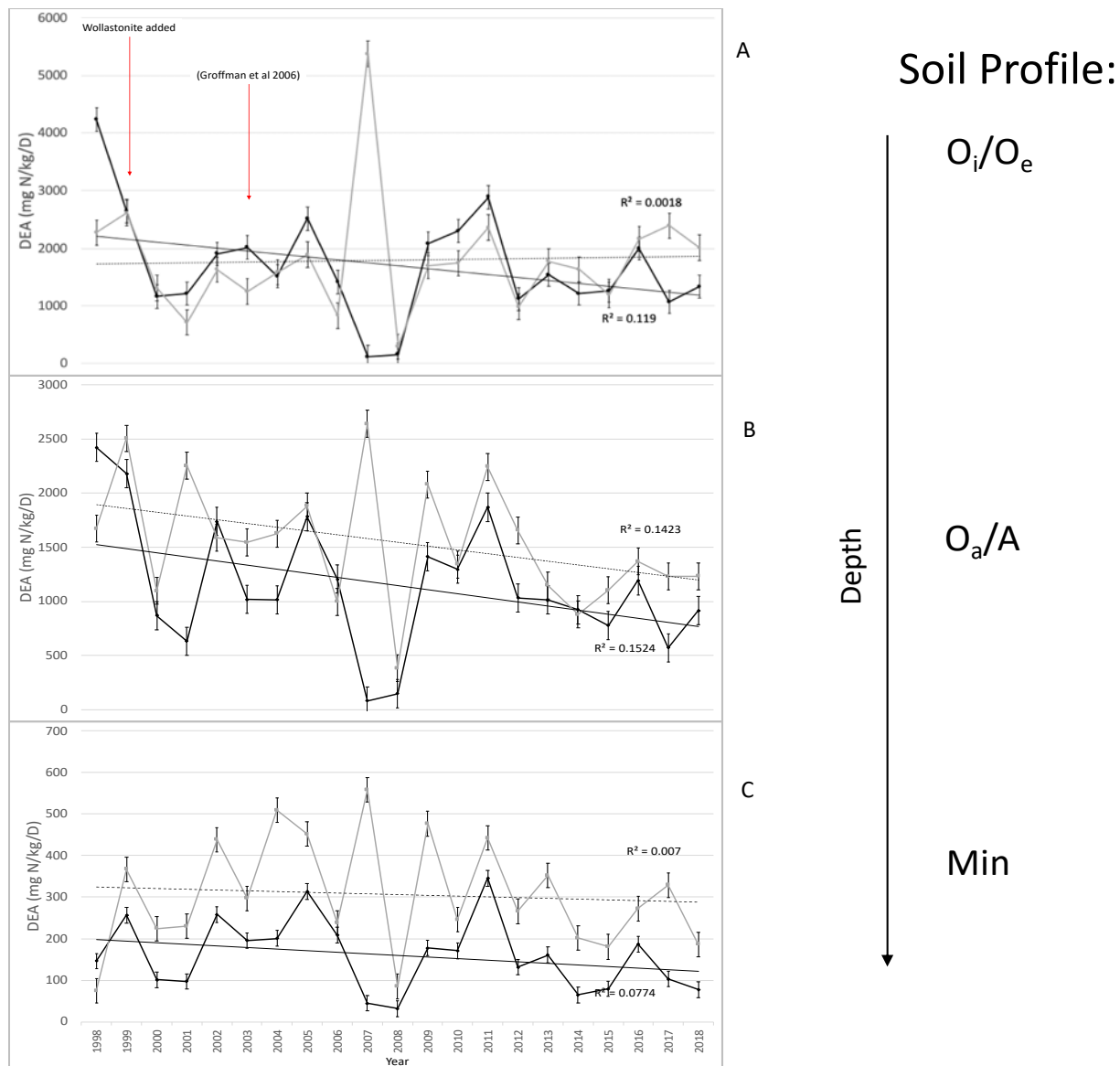
**Figure 8.** Annual average of potential net nitrification rates in the three measured soil horizons (A:  $O_i/O_e$ , B:  $O_a/A$ ; C: Mineral) in the Ca-treated (WS1; black lines) and reference (West of WS6; Bear Brook; grey lines).

### Denitrification Potential Rates

Measurements of denitrification potential in both watersheds showed higher and more variable rates in both  $O_e/O_i$  and  $O_a/A$  horizons compared to the mineral soils (Fig 9). Similar to nitrification results, denitrification potential was higher in the Ca treated watershed in  $O_a/A$  and mineral horizons (Fig 10). However, the trend of decreasing denitrification potential over time in was strongest in  $O_a/A$  horizons in both watersheds, with less steep declines in surface and mineral horizons and flatter responses in the reference area than in the Ca treated watershed (Fig 10).



**Figure 9.** Annual average denitrification potential (DEA) over 20 year period in the Ca-treated (WS1; dashed lines) and reference (West of WS6; Bear Brook; solid lines) watersheds. Arrows indicates the date of Ca treatment and the dataset in the first analysis published in 2006.



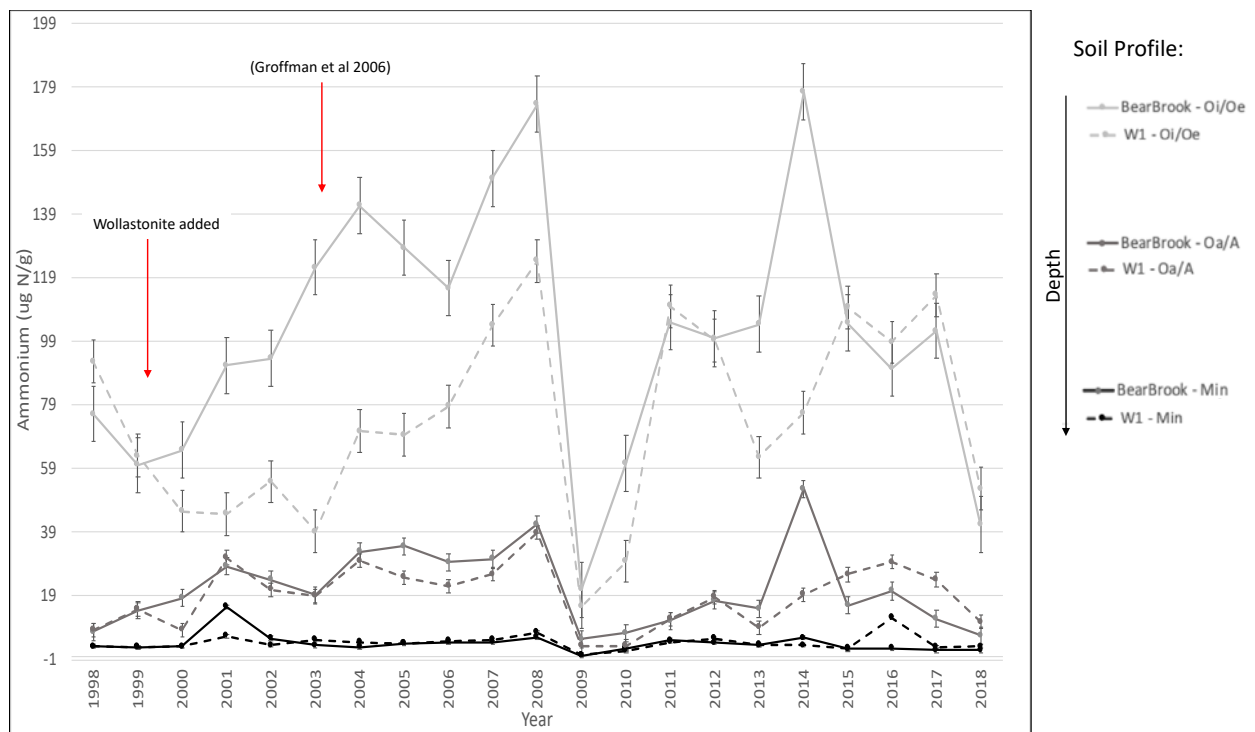
**Figure 10.** Annual average of denitrification potential (DEA) in the three measured soil horizons (A: Oi/Oe, B: Oa/A; C: Mineral) in the Ca-treated (WS1; black lines) and reference (West of WS6; Bear Brook; grey lines).

### *Soil Inorganic Nitrogen Concentrations*

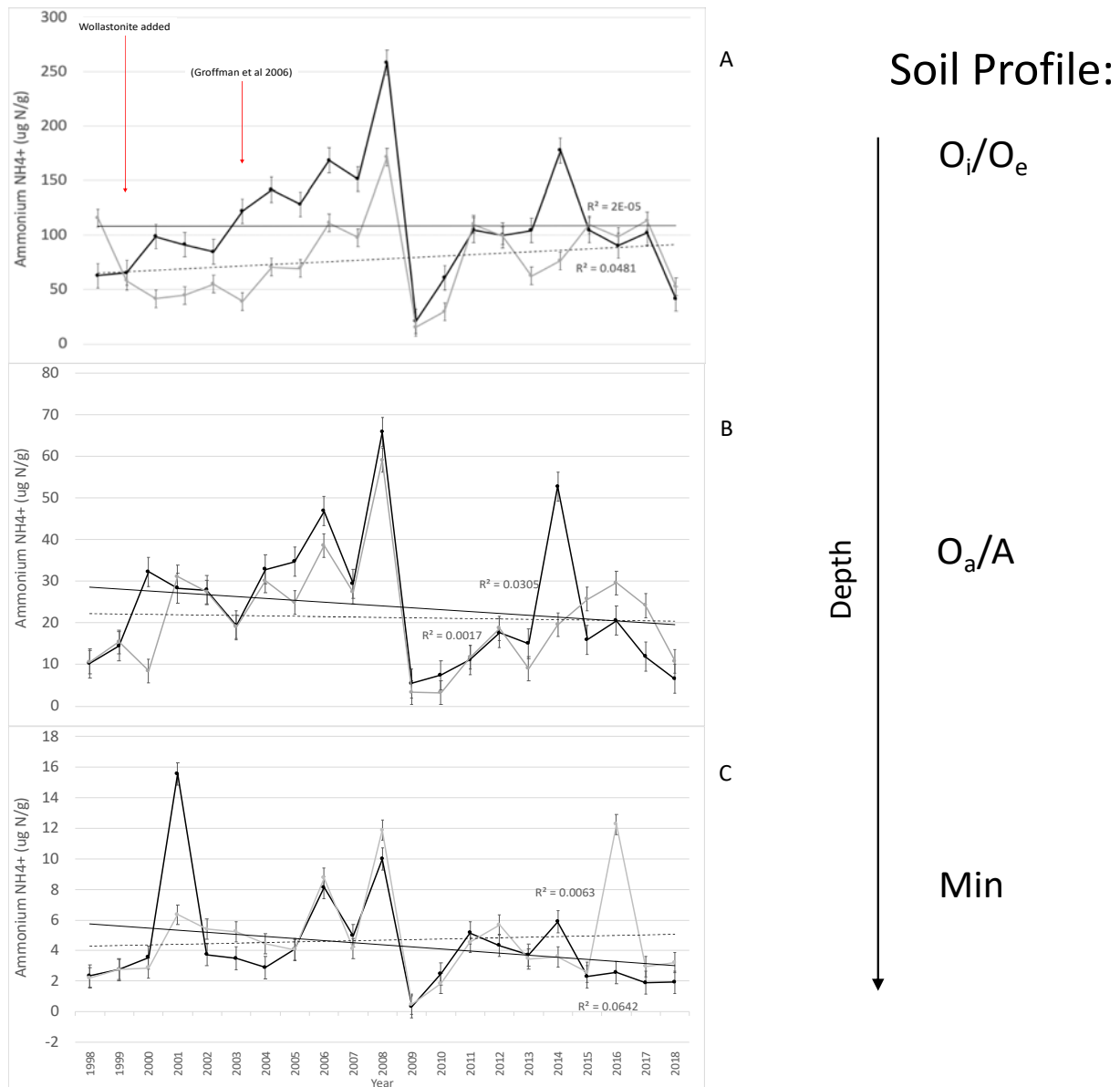
KCl-extractable soil inorganic nitrogen (ammonium and nitrate) are N forms readily available for biological uptake and represent a snapshot of the net effects of soil microbial N transformations. Ammonium concentrations in both watersheds are highest and most variable at the surface and decrease with depth (Fig 11). After the Ca addition, ammonium concentrations are higher in the reference area until about 2009, when trends began to overlap (Fig 12).

Soil nitrate concentrations were highest in two surface horizons compared to mineral soils in both the reference area and the Ca treated watershed (Fig 13). In the Ca treated watershed, nitrate concentrations tended to increase throughout the time series, while flat or slightly negative trends were seen in the reference area across all soil horizons (Fig 14).

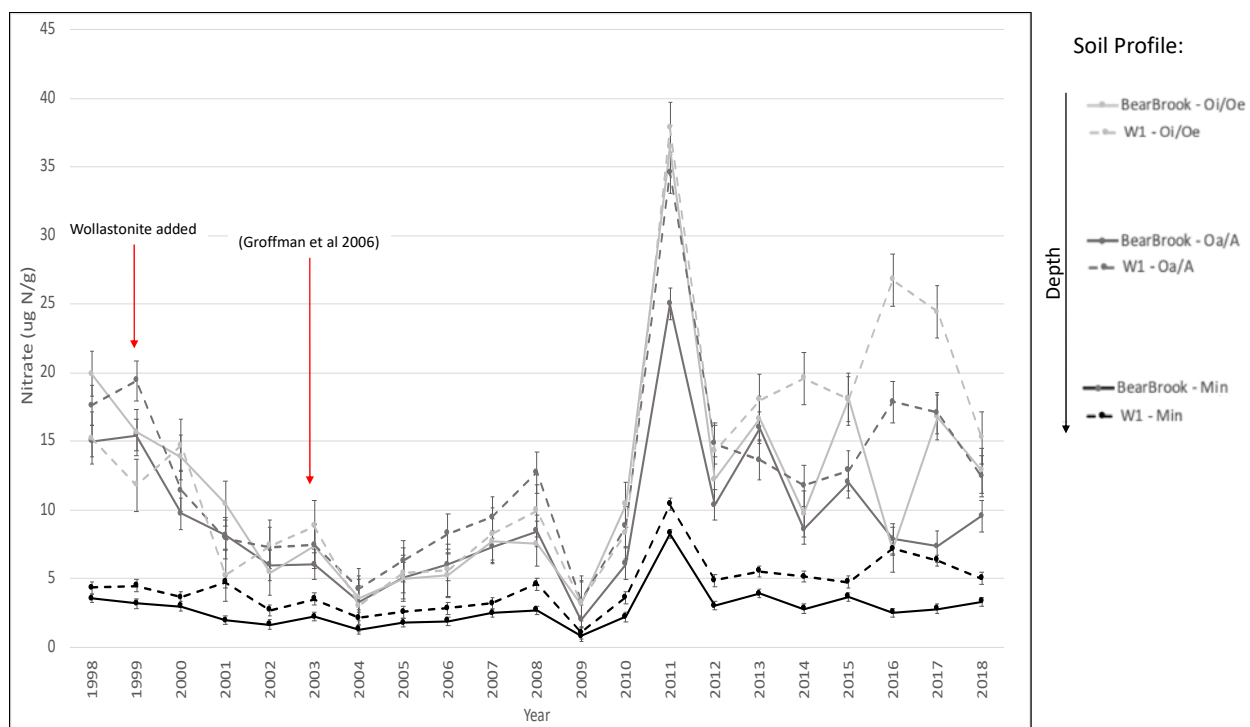




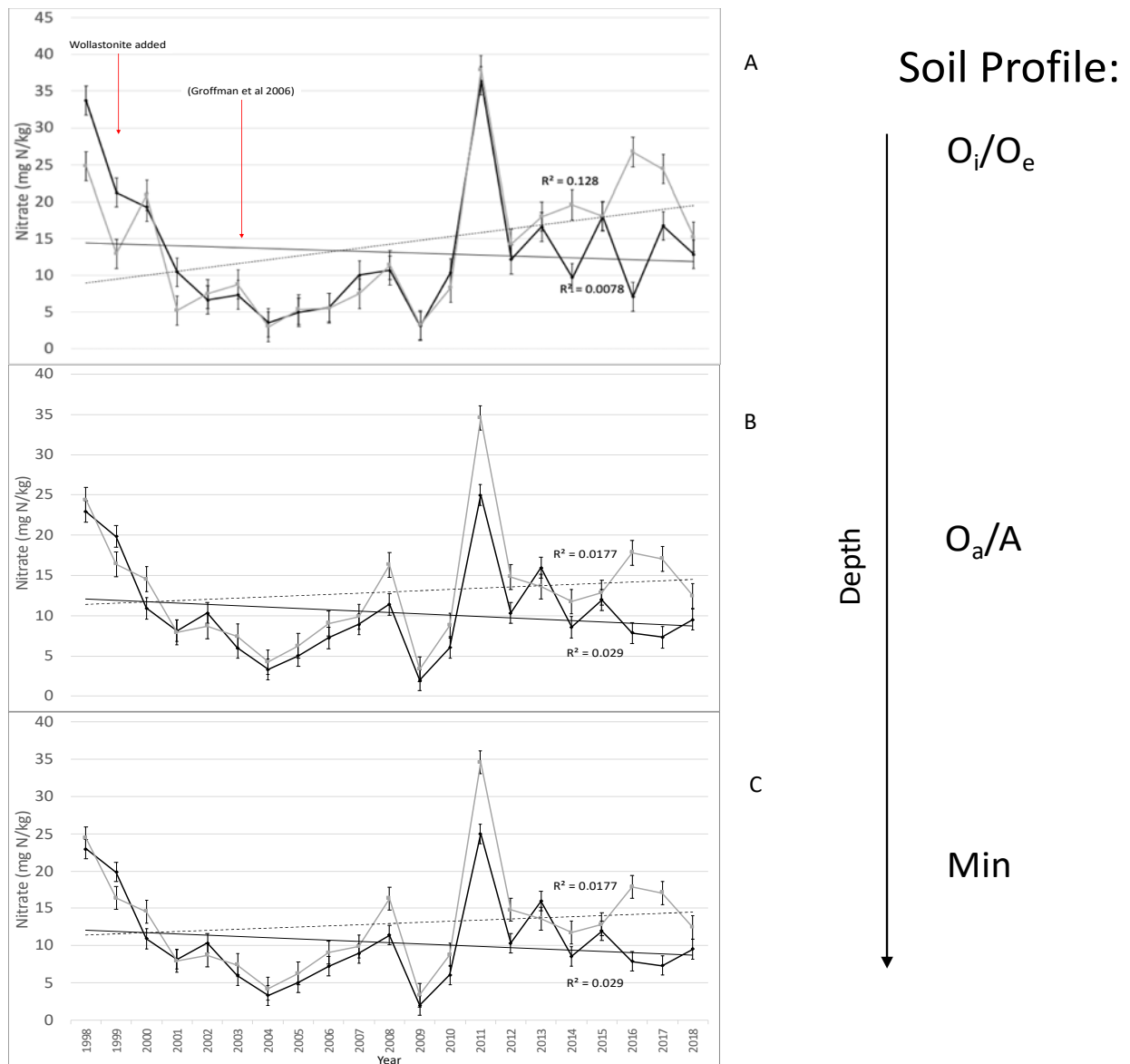
**Figure 11.** Annual average ammonium concentrations over 20 year period in the Ca-treated (WS1; dashed lines) and reference (West of WS6; Bear Brook; solid lines) watersheds. Arrows indicates the date of Ca treatment and the dataset in the first analysis published in 2006.



**Figure 12.** Annual average of 2M KCl-extractable ammonium in the three measured soil horizons (A: Oi/Oe, B: Oa/A; C Mineral) in the Ca-treated (WS1; black lines) and reference (West of WS6; Bear Brook; grey lines).



**Figure 13.** Annual average of 2M KCl-extractable nitrate over 20 year period in the Ca-treated (WS1; dashed lines) and reference (West of WS6; Bear Brook; solid lines) watersheds. Arrows indicates the date of Ca treatment and the dataset in the first analysis published in 2006.

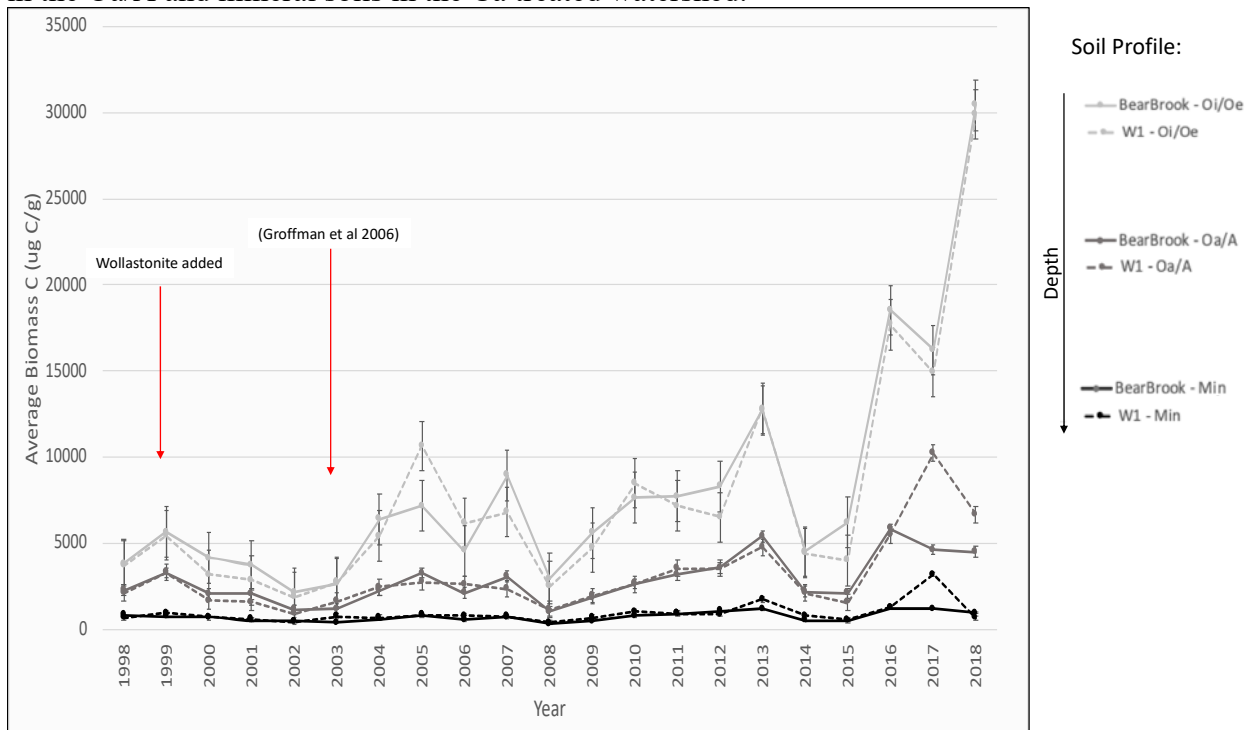


**Figure 14.** Annual average of 2M KCl-extractable nitrate in the three measured soil horizons (A: Oi/Oe, B: Oa/A; C: Mineral) in the Ca-treated (WS1; black lines) and reference (West of WS6; Bear Brook; grey lines).

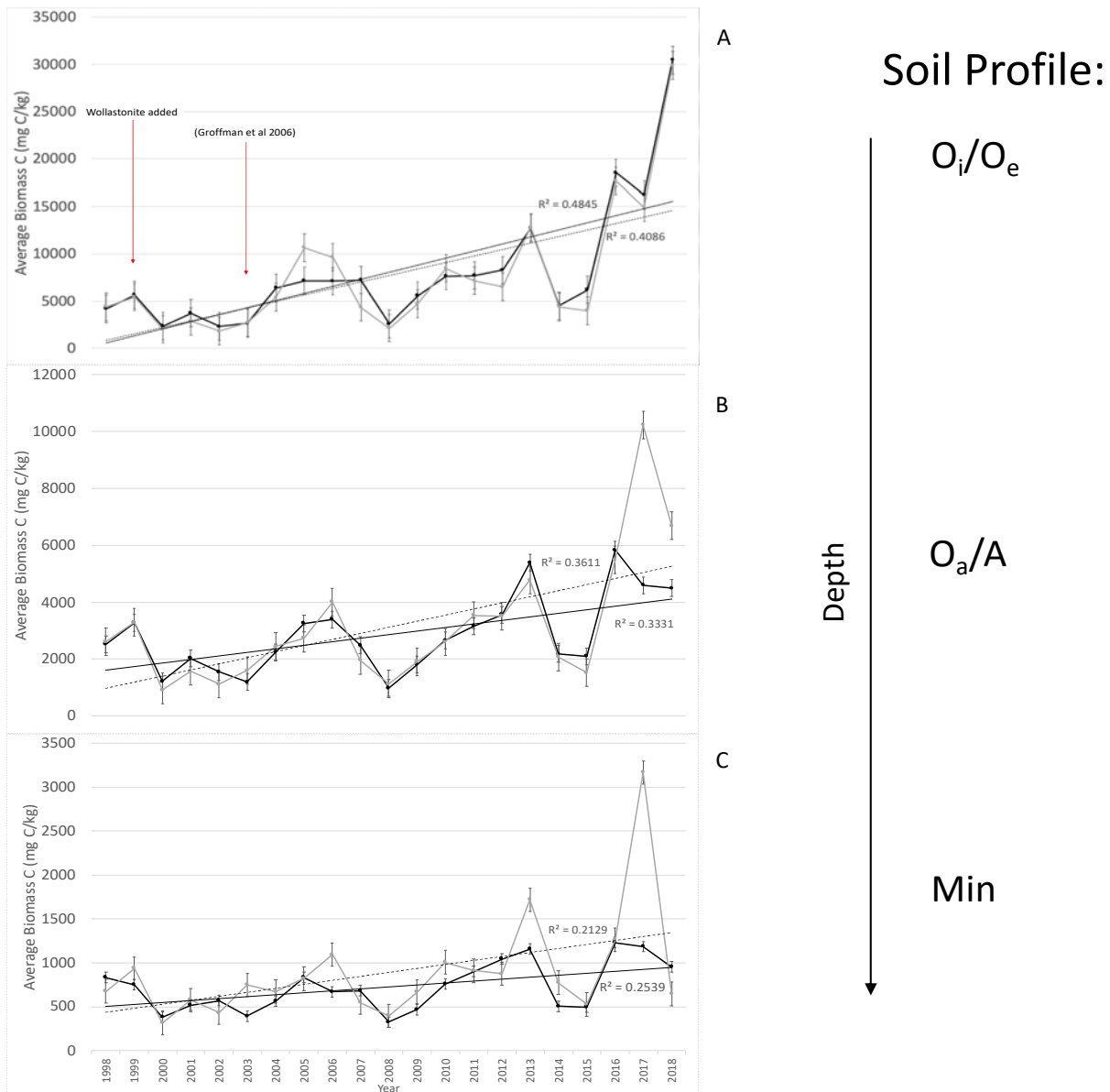
### Microbial Biomass C

In both watersheds, microbial biomass C was highest at the surface and decreased with depth (Fig 15). In the Oe/Oi horizons, there is an initially slow and progressively steep increase over time in both watersheds (Fig 15). Deeper soil horizons showed less temporal variability, but similar trends. Microbial biomass C experienced an increase in recent years that is more apparent

in the Oa/A and mineral soils in the Ca treated watershed.



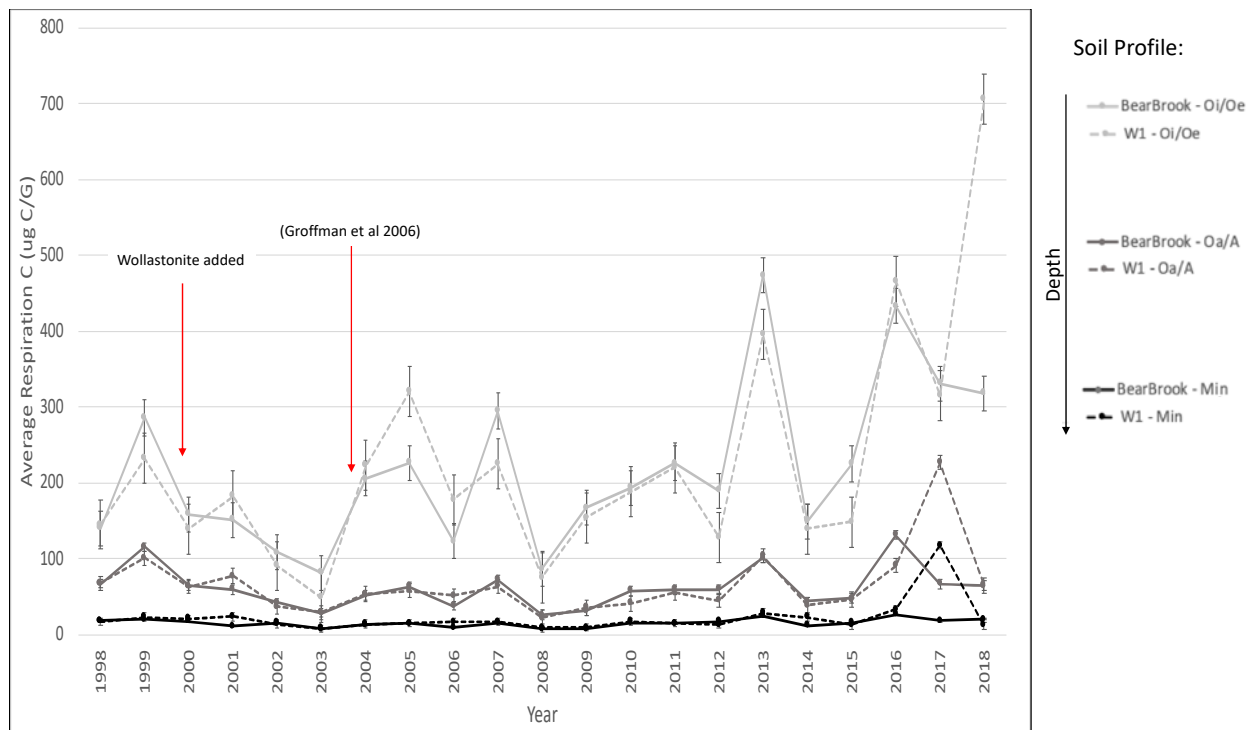
**Figure 15.** Annual average of microbial biomass C over 20 year period in the Ca-treated (WS1; dashed lines) and reference (West of WS6; Bear Brook; solid lines) watersheds. Arrows indicates the date of Ca treatment and the dataset in the first analysis published in 2006.



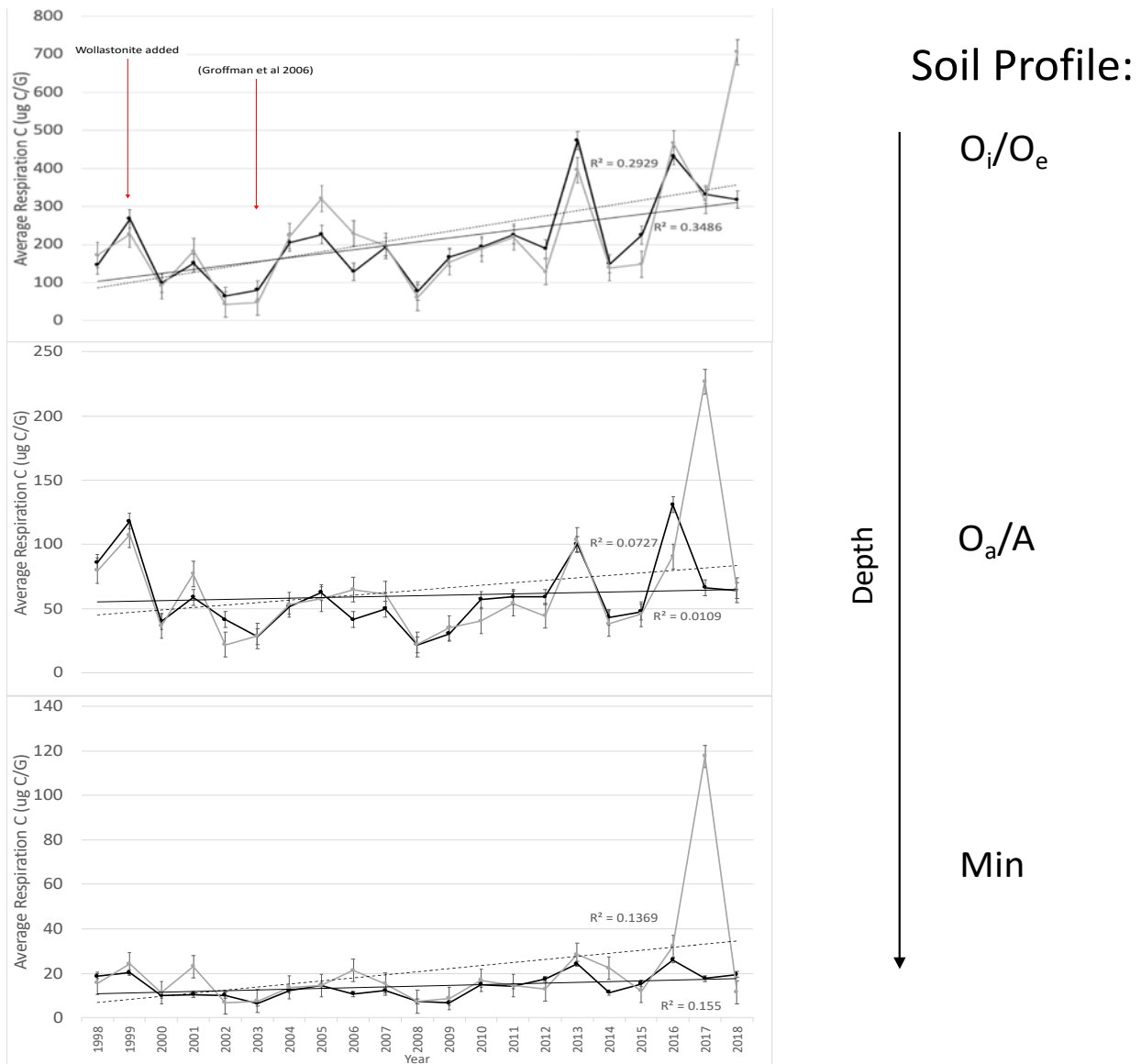
**Figure 16.** Annual average of microbial biomass C in the three measured soil horizons (A: Oi/Oe, B: Oa/A; C: Mineral) in the Ca-treated (WS1; black lines) and reference (West of WS6; Bear Brook; grey lines).

### Microbial Respiration

Soil microbial respiration was highest and most variable over time in surface soils and decreased with depth in both watersheds (Fig. 15). There were stronger increasing trends in microbial respiration over time in all horizons in the Ca treated watershed compared to the reference area (Fig. 16).



**Figure 15.** Annual average of microbial respiration C over 20 year period in the Ca-treated (WS1; dashed lines) and reference (West of WS6; Bear Brook; solid lines) watersheds. Arrows indicates the date of Ca treatment and the dataset in the first analysis published in 2006.

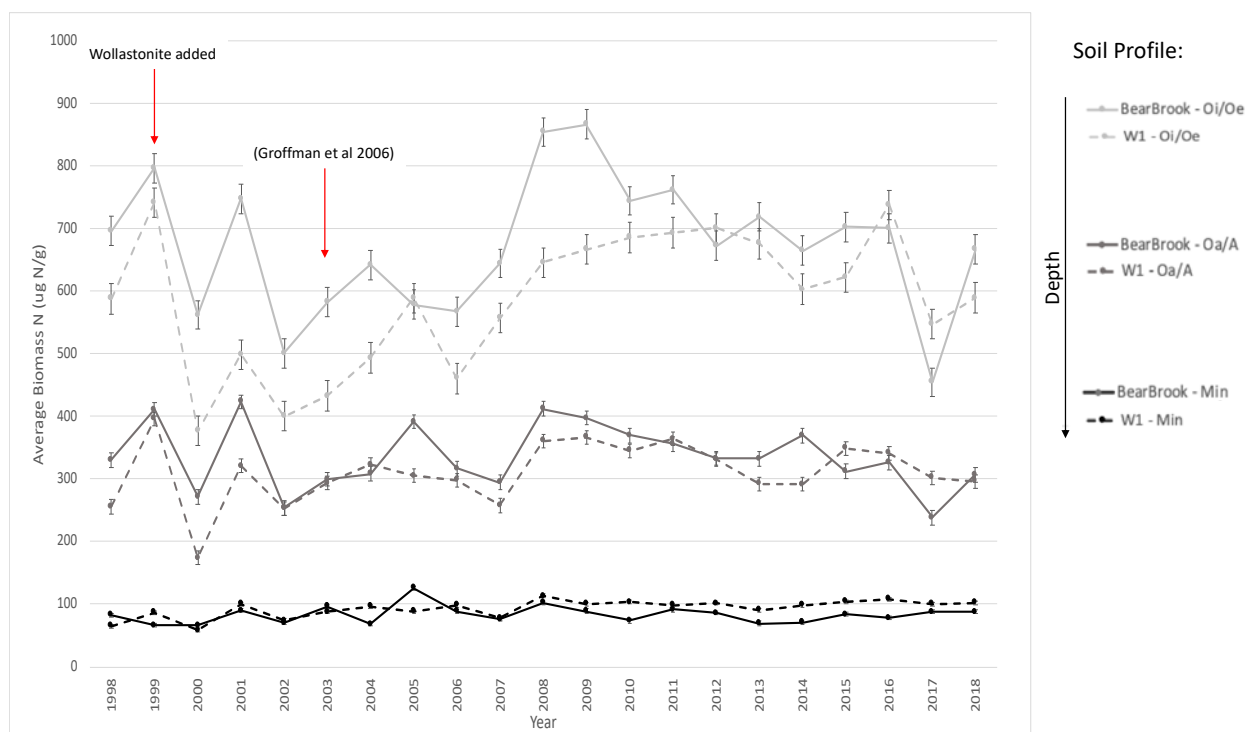


**Figure 17.** Annual average of microbial respiration C in the three measured soil horizons (A :  $O_i/O_e$ , B:  $O_a/A$ ; C Mineral) in the Ca-treated (WS1; black lines) and reference (West of WS6; Bear Brook; grey lines).

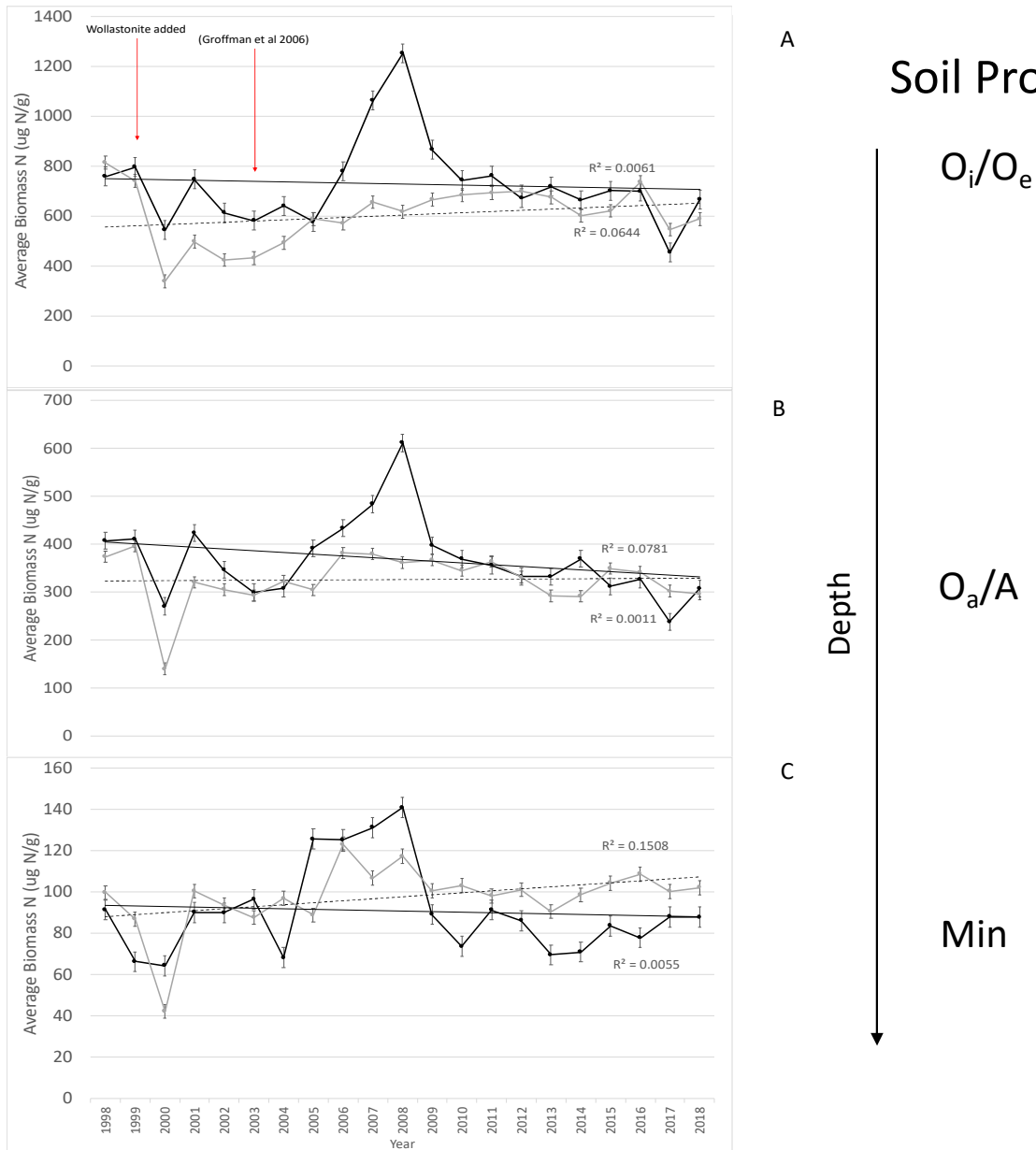
### Microbial Biomass N

Microbial biomass N also showed strong differences by soil horizon, with higher values in surface soils that decreased with depth (Fig. 18). The temporal trends in the Ca treated versus reference watersheds also differed by soil horizon, with pronounced lower microbial biomass N in the Ca treated watershed that disappeared in more recent years in both the  $O_i/O_e$  and  $O_a/A$  soils, driven in part by a decreasing trend in the reference area, whereas in the mineral soil, a divergence was seen over time, driven by increases in the Ca treated watershed (Fig 19).





**Figure 18.** Annual average of microbial biomass N over 20 year period in the Ca-treated (WS1; dashed lines) and reference (West of WS6; Bear Brook; solid lines) watersheds. Arrows indicates the date of Ca treatment and the dataset in the first analysis published in 2006.



**Figure 19.** Annual average of microbial biomass N in the three measured soil horizons (A : Oi/Oe, B: Oa/A; C Mineral) in the Ca-treated (WS1; black lines) and reference (West of WS6; Bear Brook; grey lines).

## Discussion

Observations of enhanced forest growth in the treatment watershed due to the Ca addition led to predictions of a “tightening N cycle” (Groffman and Fisk 2011; Rosi-Marshall et al 2016), whereby trees accumulate N in biomass and prevent its export from the watershed turning it into an N sink. This prediction arises from three assumptions of watershed science: higher primary production tends to retain more limiting nutrients (Vitousek and Reiners 1975; Rosi-Marshall et al. 2016); forest biomass should accumulate N when N enrichment increases, until plant demand

is saturated (Aber et al. 1989; Rosi-Marshall et al. 2016); and undisturbed and unpolluted ecosystems strongly retain inorganic nitrogen (Hedin et al. 1995; Rosi-Marshall et al. 2016).

From the long term record of soil pH, we can infer the migration of Ca throughout the soil profile in the Ca treated watershed, with an apparent dissipation of the effect of Ca on soil pH over time. It is most likely due to plant uptake, which is consistent with tree growth and rebound in tree health after the Ca addition (Groffman et al. 2006). Soil pH in both watersheds is slowly converging, with the Ca treated watershed decreasing from initial elevated values.

Nitrogen mineralization, nitrification, and denitrification potential all showed an interesting switch in the last decade, in which rates measured in the Ca treated watershed started to exceed values in the reference area. Within the soil profile, surface soils were generally more dynamic than the deeper soils. Interactions between microbial C and N cycling and chemical processes like organic matter stabilization may play an important role (Minick et al. 2017). Initial results for the 2018 NFARM data (analyzed on the NFARM in Millbrook, NY 2018) show low rates of denitrification, but it's possible that further statistical analysis may shed light on the fate of N in these soils as well as increase our understanding of the denitrification process.

In the long term record of plot-scale soil N cycling measurements, there is no clear evidence in nitrification rates or in soil nitrate concentrations prior to 2013 that explains the increase in stream N export seen in Rosi-Marshall et al. (2016). Other ecosystem drivers may explain the sudden change from a sink to a source of N in the Ca treated watershed. It is possible that the large nitrate export in 2013 could be explained by plant response to Ca addition. Plants have been found to drive ecosystem response to environmental change and variation in watershed N exports. If there is a disruption in decomposition and root-microbe interactions, this can lead to the export of inorganic N into streamwaters, which was the case during the during soil freezing events much like the one that occurred during severe ice storm events (Fitzhugh et al 2003). Dissolved inorganic N exports resulting from this experiment match or exceed earlier reports of inorganic N losses after severe ice-storm damage within the study watershed (Rosi-Marshall et al 2016). Perhaps this high stream export can be explained by similar drivers.

Long term trends in soil N cycling in the reference area are worth highlighting. These patterns may be related to changing forest demography or regional environmental change (e.g., precipitation and temperature changes; Groffman et al. 2012). There is a trend across HBEF toward warmer and wetter conditions, which could also affect plant nutrient uptake and soil N processes (Groffman et al. 2012). While soil gravimetric water content at the time of sampling showed no consistent long term trend or difference by watershed, environmental factors operating at different scales may be important drivers of soil N turnover (Groffman et al. 1989).

Effects of the Ca addition on the nitrogen cycle are complex and interacting with other environmental factors, as seen in the long term trends in the reference area alone. Complexity is also apparent in the different aspects of the nitrogen cycle that exhibit different responses to the calcium treatment and other abiotic environmental variables. There was no strong, consistent pattern in the N cycling variables in response to pH. While patterns may be driven more by environmental variables than the Ca treatment, understanding the competition for N among plants and microbes, and within the soil microbial community, remains an important consideration.

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## Supplementary Materials

Table S1. Yearly summer averages of nitrogen cycling variables in both Ca treated (WS1) and reference (West of WS6) plots.

Row Labels	Column Labels		Avg of MIN (ug N/g)		Avg of NIT (ug N/g)		Avg of DEA (ug N/g)		Avg of NH4 (ug N/g)		Avg of BION (ug N/g)		Avg of BIOC (ug C/g)		Avg of RESPC (ug C/g)	
	Avg of NO3 (ug N/g)	W1	BearBrook	W1	BearBrook	W1	BearBrook	W1	BearBrook	W1	BearBrook	W1	BearBrook	W1	BearBrook	W1
1998	33.71	24.77	36.94	33.92	5.73	3.11	4232.97	2274.99	62.89	115.59	759.21	814.53	4191.39	4393.76	146.31	172.51
1999	21.24	12.92	40.85	30.94	5.72	4.98	2647.96	2617.70	65.53	58.12	796.58	741.76	5646.79	5442.15	267.58	226.70
2000	19.30	20.89	19.08	14.56	7.65	6.08	1159.13	1307.57	98.86	41.82	545.03	340.18	2343.71	2018.59	98.50	90.76
2001	10.43	5.22	22.45	11.64	0.82	7.83	1212.12	711.04	91.48	44.74	747.61	499.14	3719.06	2843.54	151.07	182.45
2002	6.64	7.47	8.48	9.25	3.04	6.53	1905.17	1629.85	84.91	55.26	613.80	425.35	2313.04	1839.02	64.74	42.82
2003	7.37	8.77	13.94	9.13	4.69	5.07	2017.56	1246.62	122.30	39.27	582.20	432.82	2664.31	2729.90	80.94	48.41
2004	3.56	3.00	14.14	8.24	4.06	4.55	1514.82	1582.72	141.69	70.81	642.00	493.65	6388.28	5415.02	205.67	232.51
2005	5.00	5.36	19.26	12.21	5.47	8.08	2516.23	1888.64	128.43	69.63	578.63	589.20	7164.84	10656.82	226.23	320.50
2006	5.63	5.51	5.60	5.68	21.10	13.36	1413.94	829.60	168.59	111.25	780.67	572.53	7112.79	9644.70	128.28	228.95
2007	10.04	7.49	22.97	16.75	7.37	6.92	109.74	5376.18	151.34	97.78	1063.49	656.92	7238.70	4338.56	193.81	196.63
2008	10.67	11.39	23.48	5.07	3.39	5.15	151.22	287.46	258.46	171.52	1253.53	618.57	2591.60	2141.06	77.34	60.81
2009	3.11	3.24	30.99	23.87	6.10	6.38	2079.68	1694.04	20.97	15.56	866.54	666.78	5587.56	4712.46	166.86	153.57
2010	10.36	8.31	17.18	17.23	5.35	6.18	2303.15	1742.04	60.73	29.88	744.27	685.73	7631.51	8474.27	193.20	187.75
2011	36.40	37.83	3.70	4.37	15.61	11.35	2886.76	2361.35	105.04	110.11	762.05	693.46	7714.72	7162.87	225.67	219.72
2012	12.17	14.27	12.09	10.35	3.92	4.52	1118.34	980.98	99.98	99.41	672.92	700.43	8285.23	6499.65	189.31	127.82
2013	16.61	17.99	13.95	17.95	5.91	6.39	1539.88	1773.96	104.47	62.63	718.94	676.08	12715.90	12816.76	473.35	395.48
2014	9.70	19.56	13.49	16.40	4.27	5.02	1212.48	1632.58	177.44	76.57	664.93	603.28	4503.43	4398.21	149.32	138.99
2015	18.06	18.03	19.85	13.85	7.35	6.66	1258.82	1191.05	104.83	109.76	702.96	622.17	6199.72	3990.27	224.95	148.13
2016	7.08	26.73	15.76	17.57	4.74	7.85	1996.22	2163.29	90.51	98.75	700.47	737.84	18522.96	17682.24	432.85	465.94
2017	16.75	24.40	13.75	14.87	4.62	5.73	1064.16	2395.47	102.36	113.59	454.63	547.19	16218.32	14914.75	331.30	315.32
2018	12.85	15.23	13.33	10.76	5.58	5.94	1331.34	2009.22	41.40	52.71	666.66	589.41	30442.40	29888.57	318.14	705.64

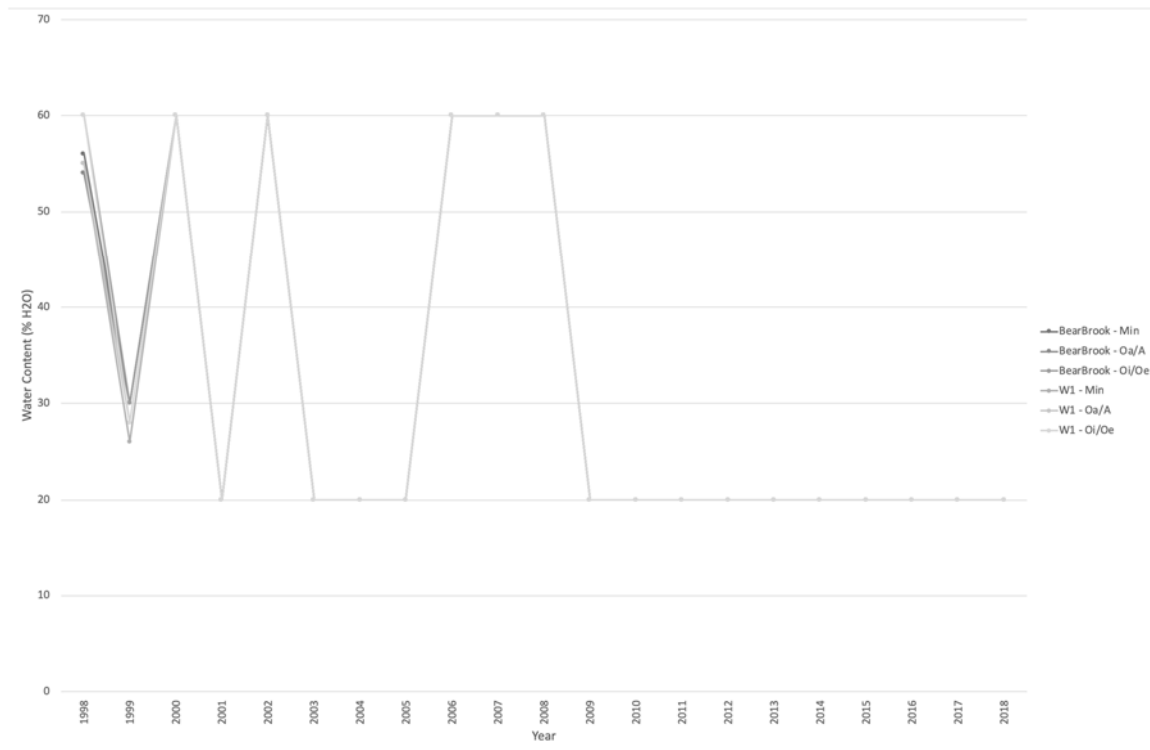


Figure S1. Water content of the soil in both Ca treated (WS1) and reference (West of WS6) plots.