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Soil nitrogen transformation responses to seasonal precipitation changes are regulated by changes in functional microbial abundance in a subtropical forest

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Table S1. Primers and thermal profiles used for the real-time PCR quantification of functional genes responsible for nitrification and denitrification.

Target gene	Primer name	Target gene length	Thermal profile	Reference
Bacterial *amoA*	*amoA* 1F/*amoA* 2R	491bp	30 s /95 °C - 35 cycles (5 s/ 95 °C - 34 s / 55 °C) - 1 min / 72 °C	Peterson *et al*. (2012)
Archaeal *amoA*	*CrenamoA* 23f/*CrenamoA* 616r	620bp	30 s /95 °C - 40 cycles (5 s/ 95 °C - 34 s / 53 °C) - 1 min / 72 °C	Levy-Booth, Prescott & Grayston (2014) Tourna *et al*. (2008)
nirk	F560-589/ R906-935	376bp	30 s /95 °C - 40 cycles (5 s/ 95 °C - 34 s / 65 °C) - 1 min / 72 °C	Chenier *et al*. (2003)
nirs	*nirS* 1F/*nirS* 3R	256bp	30 s /95 °C - 40 cycles (5 s/ 95 °C - 34 s / 65 °C) - 1 min / 72 °C	Braker, Fesefeldt & Witzel (1998) Levy-Booth & Winder (2010)
nosZ	*nosZ* 2F/*nosZ* 2R	267bp	30 s /95 °C - 40 cycles (5 s/ 95 °C - 34 s / 65 °C) - 1 min / 72 °C	Henry *et al*. (2006)

Table S2. The baseline values of soil water content (SWC), NO_3^- and NH_4^+ concentrations (mg kg^{-1}), microbial biomass carbon (MBC, mg kg^{-1}), extractable organic carbon (EOC, mg kg^{-1}), net nitrification rate (Net Nit, mg N kg^{-1} mon^{-1}), net N mineralization rate (Net N min, mg N kg^{-1} mon^{-1}) and NO_3^- and NH_4^+ leaching amounts (mg N kg^{-1} mon^{-1}) in control and precip-change (PC) plots (means and standard error, n = 4) from May to September, 2012. Differences between plots are assessed by independent sample t test, and the levels of significance are presented as: *p < 0.05.

Sampling time	plot	SWC (%)	NO_3^-	NH_4^+	MBC	TN (%)	TP (%)	EOC	TOC (%)	Net Nit	Net N min	NO_3^- Leaching	NH_4^+ Leaching
May, 2012	Control	28.4(1.0)	2.7(0.6)	4.0(0.1)	77.1(10.2)	0.23(0.02)	0.022(0.002)	173.0(29.4)	3.5(0.4)				
	PC	29.5(1.2)	2.8(0.3)	3.4(0.6)	136.9(14.8)	0.21(0.01)	0.022(0.002)	178.2(14.6)	3.2(0.2)				
Jun, 2012	Control	22.4(0.9)	1.6(0.3)	5.6(1.2)	166.3(9.6)	0.17(0.01)	0.023(0.002)	172.9(5.0)		7.2(0.8)	13.2(1.2)	8.3(0.8)*	4.3(1.1)
	PC	24.7(0.9)	1.7(0.4)	5.8(1.0)	140.8(13.5)	0.14(0.01)	0.020(0.002)	135.0(16.6)		4.9(1.3)	10.5(1.5)	5.6(0.6)	4.6(1.1)
Aug, 2012	Control	24.3(1.5)	4.8(0.4)	2.5(0.6)	215.3(14.0)	0.20(0.01)	0.026(0.002)	219.6(12.6)	3.4(0.3)				
	PC	26.4(0.7)	3.0(0.7)	3.4(0.8)	152.8(6.0)	0.14(0.02)	0.021(0.002)	177.9(13.3)	2.7(0.2)				
Sep, 2012	Control	24.5(1.1)	2.9(0.5)	3.3(1.0)	190.9(31.0)			207.2(3.1)		0.9(0.9)	4.9(2.6)	2.8(0.1)	3.2(1.1)
	PC	22.2(0.9)	5.7(1.3)	1.4(0.3)	230.9(38.6)			200.7(22.9)		5.8(1.4)	6.3(2.2)	3.0(0.2)	2.6(0.4)
Mean	Control	25.0(1.0)	3.0(0.1)	3.8(0.6)	162.4(6.4)	0.20(0.01)	0.024(0.002)	193.2(12.0)	3.5(0.3)	4.1(0.8)	9.0(1.9)	5.6(0.4)	3.7(1.1)
	PC	26.9(0.8)	3.3(0.3)	3.5(0.4)	165.3(17.4)	0.16(0.01)	0.021(0.002)	172.9(13.7)	3.0(0.2)	5.3(1.2)	8.4(1.2)	4.3(0.4)	3.6(0.7)

Table S3. Results from repeated-measures ANOVA demonstrating the effects of precip-change (PC) and sampling time on all measured soil parameters in 2013 and 2014; within-factor effects are plotted identity (*n* = 4); the significance levels are presented as: *$p < 0.05$.

		Dry season			Wet season		
	Variable	PC	Time	PC × Time	PC	Time	PC × time
		F (1)	F (5)	F (1, 5)	F (1)	F (3)	F (1, 3)
2013	SWC (%)	33.23*	6.36*	2.09	3.33	3.48*	1.00
	NO_3^- (mg kg^{-1})	27.40*	2.74	9.84	0.11	14.53*	2.23
	NH_4^+ (mg kg^{-1})	0.01	18.66*	0.90	2.34	3.05	0.38
	$NO_3^- + NH_4^+$	73.20*	15.84*	1.22	2.91	13.00*	1.27
	$NH_4^+ : NO_3^-$	12.43*	74.73*	15.28	0.59	2.78	0.57
	EOC (mg kg^{-1})	1.90	11.17*	5.31*	6.43*	13.61*	6.51*
	MBC (mg kg^{-1})	0.36	1.10	0.41	0.06	1.47	1.24
2014	SWC (%)	24.16*	59.74*	1.58	8.67*	6.10*	1.10
	NO_3^- (mg kg^{-1})	31.65*	33.29*	0.69	10.76*	21.52*	8.76*
	NH_4^+ (mg kg^{-1})	6.87*	107.10*	3.02	3.70	12.20*	0.90
	$NO_3^- + NH_4^+$	13.02*	39.31*	1.15	10.29*	4.53*	2.96
	$NH_4^+ : NO_3^-$	1.16	11.86	1.65	5.07	47.81*	3.37
	EOC (mg kg^{-1})	0.62	69.48*	0.55	0.01	5.22*	1.06
	MBC (mg kg^{-1})	1.76	15.516*	1.07	0.05	4.334*	0.58

Table S4. Results from repeated-measures ANOVA demonstrating the effects of precip-change (PC) and sampling time on nitrogen transformation rates (Net Nit: net nitrification rate; Net N min: net N mineralization rate) and inorganic nitrogen leaching contents in 2013 and 2014; within-factor effects are plotted identity (n = 4). The measurement N_2O was repeated for 12 times during each season, thus the df values were different (i.e., PR: df = 1; Time: df = 11; PR × Time: df = 1, 11). The significance levels are presented as: *p < 0.05.

		Dry season			Wet season		
	Variable	PC	Time	PC × Time	PC	Time	PC × Time
		F (1)	F (2)	F (1, 2)	F (1)	F (1)	F (1, 1)
2013	Net nit (mg N kg^{-1} mon^{-1})	3.97	11.09*	0.05	55.60*	5.07	0.00
	Net N min (mg N kg^{-1} mon^{-1})	4.00	38.10*	1.11	33.24*	5.23	0.00
	NO_3^- leaching(mg N kg^{-1} mon^{-1})	2.48	6.84*	1.49	32.23*	6.80*	0.02
	NH_4^+ leaching(mg N kg^{-1} mon^{-1})	2.14	12.15*	0.51	1.08	12.48*	1.95
	(NO_3^- + NH_4^+) leaching	4.42	4.48*	2.02	30.56*	7.63*	0.04
	N_2O (ug N-N_2O m^{-2} h^{-1})	0.08	7.63*	1.92	0.54	4.31*	0.90
2014	Net nit (mg N kg^{-1} mon^{-1})	35.19*	186.20*	19.67*	0.11	0.01	0.83
	Net N min (mg N kg^{-1} mon^{-1})	35.51*	103.70*	3.19	0.48	0.41	1.16
	NO_3^- leaching(mg N kg^{-1} mon^{-1})	7.39*	484.40*	9.45*	0.08	0.54	1.07
	NH_4^+ leaching(mg N kg^{-1} mon^{-1})	1.27	18.08*	0.74	0.01	0.50	0.49
	(NO_3^- + NH_4^+) leaching	7.14*	370.90*	6.32	0.04	0.52	2.50
	N_2O (ug N-N_2O m^{-2} h^{-1})	0.41	8.45*	1.47	0.02	12.16*	0.24

Table S5. Results from repeated-measures ANOVA demonstrating the effects of precip-change (PC) and sampling time on functional microbial gene abundance (copy numbers per gram of dry soil) in 2013 and 2014; within-factor effects are plotted identity ($n = 4$); the significance levels are presented as: *$p < 0.05$.

		Dry season			Wet season		
	Variable	PC	Time	PC × Time	PC	Time	PC × Time
		F (1)	F (3)	F (1, 3)	F (1)	F (3)	F (1, 3)
2013	*amoA*	0.12	11.73*	3.84*	0.85	23.22*	11.32*
	nirK	1.09	47.35*	1.39	1.42	33.80*	5.08*
	nirS	77.87*	66.89*	13.06*	0.26	27.84*	5.85*
	nosZ	10.71*	11.31*	2.78	1.51	5.30*	6.60*
		F (1)	F (5)	F (1, 5)	F (1)	F (3)	F (1, 3)
2014	*amoA*	43.79*	25.79*	1.01	1.27	10.52*	1.78
	nirK	0.00	14.35*	0.44	0.10	0.37	1.07
	nirS	0.24	26.83*	1.74	0.00	4.13*	0.82
	nosZ	3.74	1.48	1.94	0.15	9.18*	1.44

Fig. S1 Deployment of the precipitation manipulating facilities including supporting structures, rainout shelters and water addition subsystems.

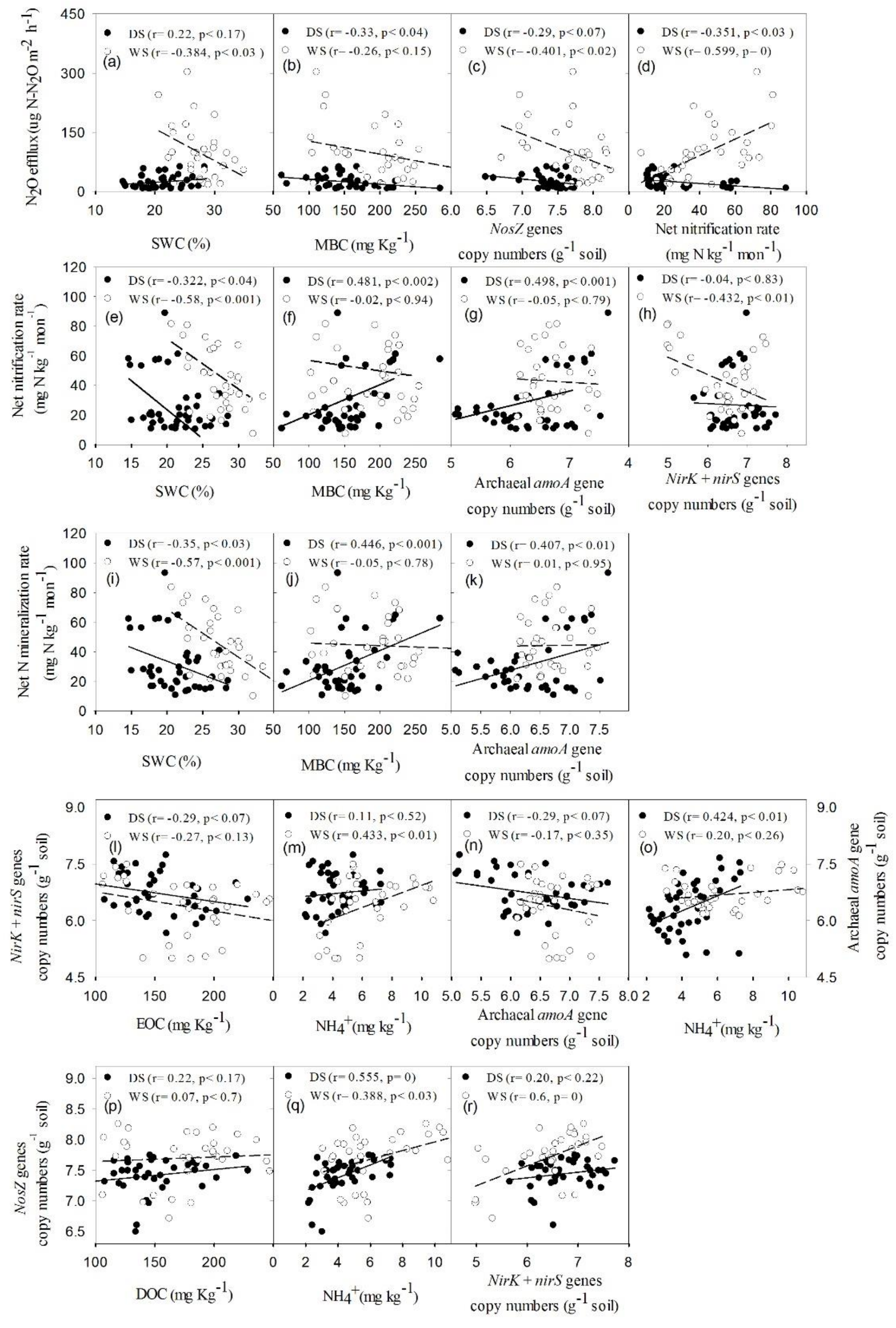

Fig. S2. Correlations for the variables showing significantly direct relationships in the structure equation models (SEM). Coefficients and *p* values of correlations for dry season (DS) and wet season (WS) were calculated, respectively. Data from both precip-redistribution and control plots were included in the bivariate plots (DS: $n = 40$, WS: $n = 32$), with log transformations for the genes abundance.

Reference

Braker G, Fesefeldt A, Witzel KP (1998) Development of PCR primer systems for amplification of nitrite reductase genes (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples. *Applied and Environmental Microbiology*, **64**, 3769-3775.

Chenier MR, Beaumier D, Roy R, Driscoll BT, Lawrence JR, Greer CW (2003) Impact of seasonal variations and nutrient inputs on nitrogen cycling and degradation of hexadecane by replicated river biofilms. *Applied and Environmental Microbiology*, **69**, 5170-5177.

Henry S, Bru D, Stres B, Hallet S, Philippot L (2006) Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, *narG, nirK*, and *nosZ* genes in soils. *Applied and Environmental Microbiology*, **72**, 5181-5189.

Levy-Booth DJ, Prescott CE, Grayston SJ (2014) Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. *Soil Biology and Biochemistry*, **75**, 11-25.

Levy-Booth DJ, Winder RS (2010) Quantification of Nitrogen Reductase and Nitrite Reductase Genes in Soil of Thinned and Clear-Cut Douglas-Fir Stands by Using Real-Time PCR. *Applied and Environmental Microbiology*, **76**, 7116-7125.

Petersen DG, Blazewicz SJ, Firestone M, Herman DJ, Turetsky M, Waldrop M (2012) Abundance of microbial genes associated with nitrogen cycling as indices of biogeochemical process rates across a vegetation gradient in Alaska. *Environmental Microbiology*, **14**, 993-1008.

Tourna M, Freitag TE, Nicol GW, Prosser JI (2008) Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environmental Microbiology*, **10**, 1357-1364.