Supplementary information

N-cycle genes abundance determined N mineralization rate following afforestation in the Loess Plateau of China

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Table S1. Results from the redundancy analysis (RDA) to identify the conditional effects of soil properties on soil nitrogen functional genes abundance.

Name	Explains %	Contribution %	pseudo-F	*P*
MBN	53.8	60.5	15.2	**0.002**
Clay	11	12.4	3.8	**0.004**
Silt	3.3	3.7	1.1	0.348
TIN	3	3.3	1	0.408
SOC	4.2	4.8	1.6	0.164
TN	3.7	4.1	1.4	0.206
C/N	2	2.3	0.7	0.61
SM	3	3.4	1.2	0.324
BD	2.2	2.5	0.8	0.528
NO_3^-	1.6	1.8	0.5	0.712
PH	1	1.1	0.3	0.868

The conditional effects represent the proportion of variation explained by the individual environmental variables (soil properties). Significant results are shown in bold.

BD, bulk density; SOC, total soil organic C; TN, total nitrogen; C/N, the ratio of SOC and TN; TIN, total inorganic nitrogen; MBN, microbial biomass nitrogen; SM, soil moisture.

Table S2 Results of final plspm model presented in figure 4.

Number	Relationships	Direct	Indirect	Total
1	Soil physical properties →Soil chemical properties	-0.618	0.000	-0.618
2	Soil physical properties →Soil microbial community	0.061	-0.534	-0.474
3	Soil physical properties →Nitrification	-0.011	-0.561	-0.572
4	Soil physical properties →Denitrification	-0.486	-0.126	-0.611
5	Soil physical properties →Soil mineralization rate	0.144	-0.664	-0.520
6	Soil chemical properties →Soil microbial community	0.864	0.000	0.864
7	Soil chemical properties →Nitrification	0.580	0.370	0.950
8	Soil chemical properties →Denitrification	-0.932	1.199	0.267
9	Soil chemical properties →Soil mineralization rate	-0.136	0.874	0.738
10	Soil microbial community →Nitrification	0.428	0.000	0.428
11	Soil microbial community →Denitrification	0.419	0.377	0.796
12	Soil microbial community →Soil mineralization rate	-0.156	0.776	0.620
13	Nitrification →Denitrification	0.881	0.000	0.881
14	Nitrification→ Soil mineralization rate	0.928	0.420	1.348
15	Denitrification →Soil mineralization rate	0.477	0.000	0.476

Table S3. Results of final plspm model presented in figure 4.

	Type	R^2	Block Communality	Mean Redundancy	AVE
Soil physical properties	Exogenous	0.000	0.766	0.000	0.766
Soil chemical properties	Endogenous	0.382	0.791	0.302	0.791
Soil microbial community	Endogenous	0.685	0.817	0.560	0.817
Nitrification	Endogenous	0.942	0.549	0.517	0.549
Denitrification	Endogenous	0.662	0.636	0.421	0.636
Soil mineralization rate	Endogenous	0.910	1.000	0.910	1.000

Table S4 Loading coefficient between observation variables and latent variables.SPP: soil physical properities, SCP: soil chemical properities, MC: microbial community, Ni: nitrification ,De: denitrification, R_m: Soil net mineralization rate. * indicates that there is a significant relationship between latent variables.

Latent variables	Observation variables	SPP	SCP	MC	Ni	De	R_m
SPP	Bulk density	0.849	-0.599*	-0.458	-0.520	-0.302*	-0.312
	Silt	0.901	-0.496*	-0.380	-0.486	-0.729*	-0.574
SCP	Soil organic C	-0.609	0.944	0.858*	0.946*	0.675*	0.854
	Total N	-0.472	0.830	0.556*	0.682*	0.175*	0.441
MC	Shannon	-0.520	0.723	0.916	0.814*	0.712	0.789
	NMDS1	-0.325	0.775	0.892	0.837*	0.510	0.717
Ni	*amoB*	0.034	0.316	0.403	0.384	0.080	0.210*
	narH,narY,nxrB	-0.619	0.927	0.876	0.975	0.690	0.901*
De	*norC*	-0.169	0.285	0.340	0.310	0.615	0.303*
	norB	-0.663	0.533	0.678	0.669	0.946	0.864*
R_m	net mineralization rate	-0.520	0.777	0.835	0.892	0.825	1.000

Table S5. Changes of gene abundance (Nitrification genes and Denitrification genges) along with afforestation chronosequence.

	Gene	FL	RP14	RP20	RP30	RP45	*P
Nitrification	*hao*	0.62±0.09a	0b	0.85±0.22a	0.68±0.03a	0b	**0.001**
	pmoA-amoA	8.54±1.22c	12.25±0.15ab	15.07±0.59a	11.33±1.24b	13.71±0.62ab	**0.003**
	pmoB-amoB	10.32±1.25c	16.17±1.37a	14.70±1.09ab	11.06±0.70b	14.97±1.61ab	**0.027**
	pmoC-amoC	13.02±1.15b	15.74±0.53ab	19.18±1.58a	16.31±1.82ab	18.85±1.54a	0.059
	narG,narZ,nxrA	34.80±2.27c	40.35±1.64bc	52.68±2.33a	51.77±5.49ab	59.71±4.83a	**0.004**
	narH,narY,nxrB	16.59±0.34c	19.82±1.17c	24.14±1.05b	27.68±1.82ab	29.90±1.26a	**0.000**
Denitrification	*nosZ*	1.49±0.57a	0.65±0.33ab	0.63±0.27ab	0.52±0.12ab	0b	0.090
	nirk	20.09±1.33a	16.74±1.00a	20.17±3.88a	18.89±1.98a	19.92±1.79a	0.792
	norC	0.66±0.52ab	0.53±0.23ab	0.80±0.26ab	0b	1.41±0.16a	0.062
	norB	7.41±0.86d	4.72±1.49cd	9.33±0.76bc	11.91±1.44ab	13.29±1.16a	**0.003**

Table S6. Changes of gene abundance (Six nitrogen metabolism pathways) along with afforestation chronosequence.

	FL	RP14	RP20	RP30	RP45	*P
Nitrification	83.89±1.81d	104.34±2.46c	126.60±4.07ab	118.82±8.18b	137.24±4.81a	**0.000**
Denitrification	29.65±1.14ab	22.64±1.79b	30.92±4.35ab	31.63±3.73ab	34.90±0.85b	0.094
Assimilatory nitrate reduction	239.39±2.73ab	245.36±11.96a	221.56±3.14b	227.60±2.80ab	231.17±4.85ab	0.127
Dissimilatory nitrate reduction	358.73±2.54a	330.55±11.06b	322.95±3.84b	340.43±4.80ab	342.53±9.31ab	**0.045**
Ammoniation	205.87±4.32c	233.64±10.58bc	250.78±11.77ab	249.21±10.26ab	266.57±5.95a	**0.008**
Glutamine synthesis	204.34±6.02cd	197.66±3.82d	223.85±4.01bc	233.75±9.91b	259.55±7.02a	**0.000**

Fig.S1.

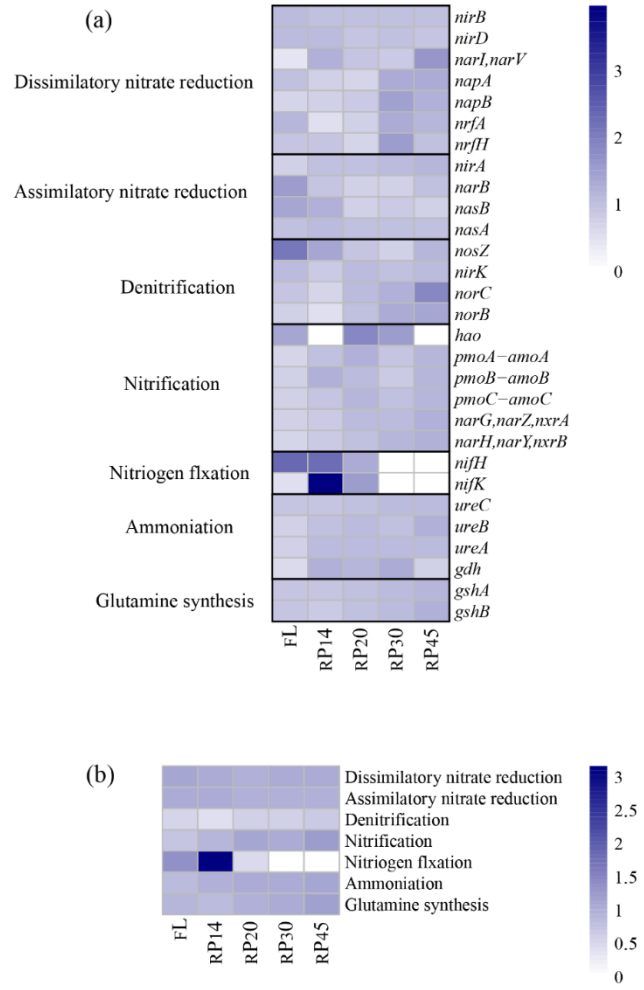

(a)
Dissimilatory nitrate reduction
Assimilatory nitrate reduction
Denitrification
Nitrification
Nitriogen flxation
Ammoniation
Glutamine synthesis
nirB
nirD
narI,narV
napA
napB
nrfA
nrfH
nirA
narB
nasB
nasA
nosZ
nirK
norC
norB
hao
pmoA−amoA
pmoB−amoB
pmoC−amoC
narG,narZ,nxrA
narH,narY,nxrB
nifH
nifK
ureC
ureB
ureA
gdh
gshA
gshB
FL
RP14
RP20
RP30
RP45
3
2
1
0
(b)
Dissimilatory nitrate reduction
Assimilatory nitrate reduction
Denitrification
Nitrification
Nitriogen flxation
Ammoniation
Glutamine synthesis
FL
RP14
RP20
RP30
RP45
3
2.5
2
1.5
1
0.5
0

Fig.S2.

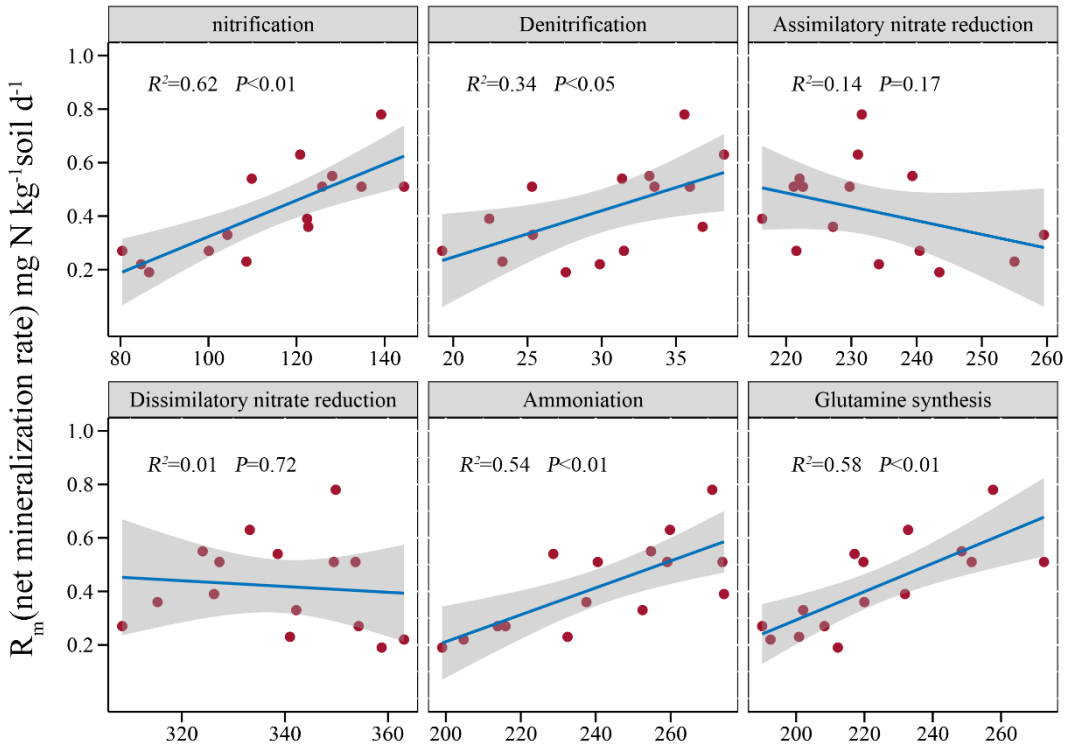

nitrification
Denitrification
Assimilatory nitrate reduction
Dissimilatory nitrate reduction
Ammoniation
Glutamine synthesis
R²=0.62 P<0.01
R²=0.34 P<0.05
R²=0.14 P=0.17
R²=0.01 P=0.72
R²=0.54 P<0.01
R²=0.58 P<0.01
Rm (net mineralization rate) mg N kg-1soil d-1

Fig.S3.

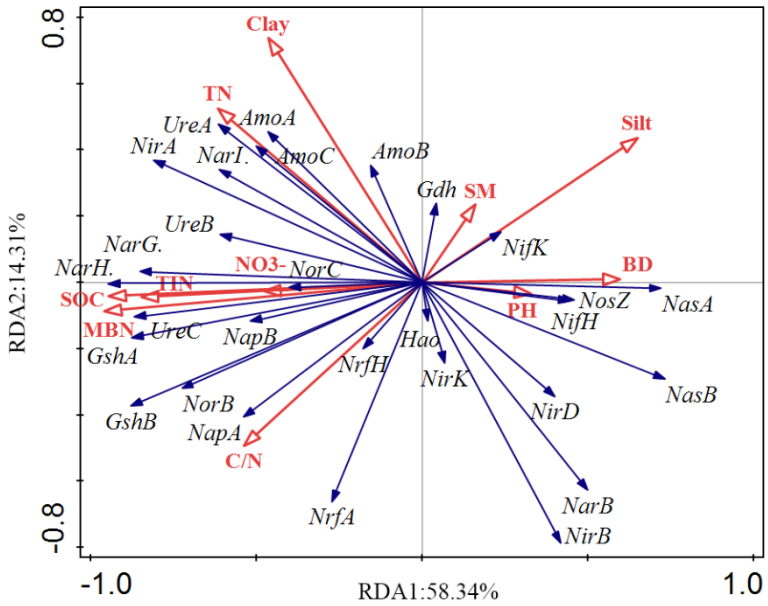
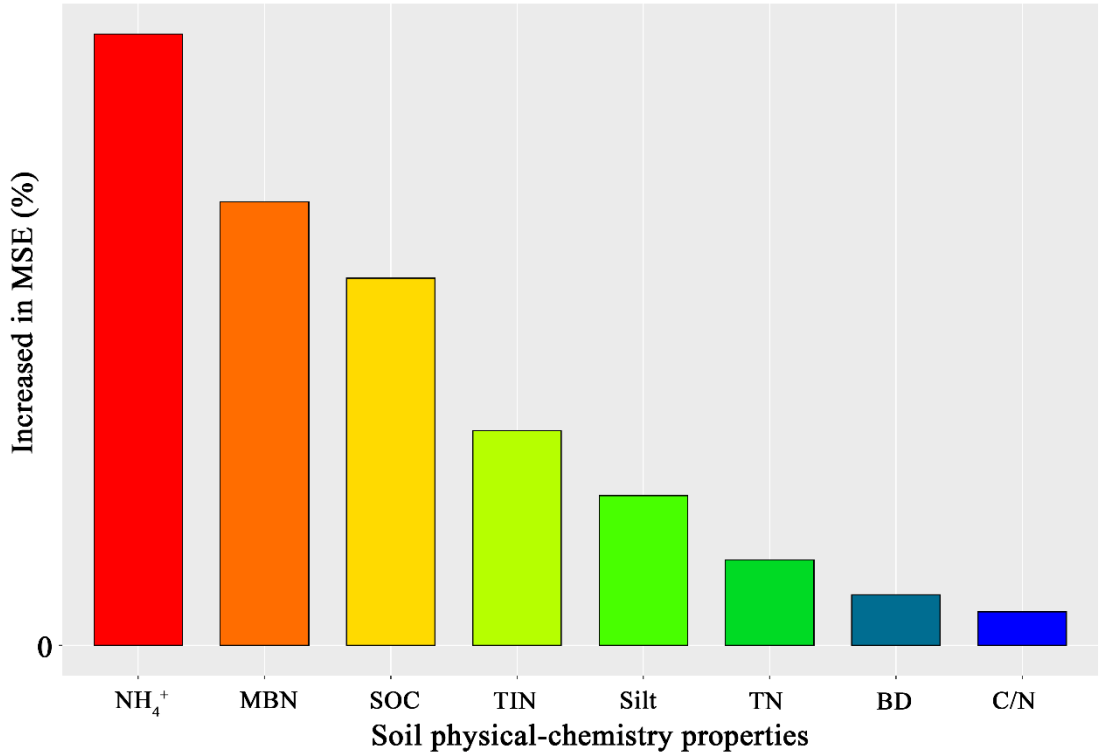

Clay
TN
UreA
AmoA
NirA
NarI.
AmoC
AmoB
Gdh
SM
Silt
UreB
NarG.
NifK
NarH.
NO3-
NorC
BD
TIN
SOC
PH
NosZ
NasA
NifH
MBN
UreC
NapB
Hao
GshA
NrfH
NirK
NasB
NorB
NirD
GshB
NapA
C/N
NrfA
NarB
NirB
0.8
-0.8
-1.0
1.0
RDA2:14.31%
RDA1:58.34%

Fig.S4.


Increased in MSE (%)
0
NH_4^+
MBN
SOC
TIN
Silt
TN
BD
C/N
Soil physical-chemistry properties