Soil Biology and Biochemistry 135 (2019) 392–395

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Short Communication

Comammox *Nitrospira* clade B contributes to nitrification in soil

Zhihui Wang[a], Yanqiang Cao[a], Xia Zhu-Barker[b], Graeme W. Nicol[c], Alan L. Wright[d], Zhongjun Jia[e], Xianjun Jiang[a,*]

[a] College of Resources and Environment, Southwest University, 2 Tiansheng Road, Beibei, Chongqing, 400715, China
[b] Biogeochemistry and Nutrient Cycling Laboratory, Department of Land, Air and Water Resources, University of California Davis, CA, 95616, USA
[c] Laboratoire Ampère, École Centrale de Lyon, Université de Lyon, Ecully, 69134, France
[d] Indian River Research & Education Center, University of Florida-IFAS, Fort Pierce, FL, 34945, USA
[e] State Key Laboratory of Soil and Sustainable Agriculture Institute of Soil Science, Chinese Academy of Sciences, Nanjing, Jiangsu, 210008, China

ARTICLE INFO

Keywords:
Comammox
Ammonia oxidizing bacteria
Ammonia oxidizing archaea
Nitrification
DNA-SIP

ABSTRACT

Comammox, one nitrifying microorganism carries out the complete oxidation of ammonia to nitrate, have been recently discovered, and are found in a wide range of environments, including soil. However, conditions under which they actually contribute to nitrification in soil have not yet been demonstrated. By $^{13}CO_2$-based DNA stable isotope probing with real-time quantitative PCR and gene sequence, we reported two uncultured strains, which are closely related to comammox *Nitrospira* clade B, autotrophically grew in both forest and paddy soils only in the absence of ammonium amendment. Furthermore, all clade B *amoA* sequences amplified from isotopically enriched genomic DNA in both soils were derived from one or two phylotypes, indicating a low diversity of active comammox strains in soils.

1. Main text

Nitrification was long considered a two-step process with ammonia oxidized to nitrite followed by oxidation to nitrate by two functionally-distinct groups: ammonia and nitrite oxidizers (Winogradsky, 1890; Bock and Wagner, 1992; Könneke et al., 2005). However, microorganisms capable of complete oxidation of ammonia to nitrate (comammox) have recently been discovered (van Kessel et al., 2015; Daims et al., 2015; Pinto et al., 2015). Comammox bacteria belong to the genus *Nitrospira*, organisms that were traditionally considered as strict nitrite-oxidizing bacteria, although recent studies demonstrate they possess considerable metabolic versatility (Daims et al., 2016; Santoro, 2016). Comammox bacteria are differentiated into two clades (A and B) based on phylogeny of genes encoding the α-subunit of ammonia monooxygenase genes (*amoA*) (Daims et al., 2015; Pjevac et al., 2017). All comammox representatives cultivated from aquatic ecosystems belong to clade A (Daims et al., 2016), and no representative enrichments or isolates have yet been obtained from soil, limiting our understanding of their contribution to nitrification. Cultivated representatives of clade A comammox are proposed to outcompete canonical ammonia oxidizers under conditions of low ammonia availability due to a higher affinity for ammonia (Kits et al., 2017; Kuypers, 2017; Palomo et al., 2018), indicating that ammonia concentrations may play a role in regulating comammox activity.

In this study, autotrophic growth of comammox bacteria during nitrification was examined in aerobic microcosms for two contrasting soils (paddy and coniferous forest) incubated with and without added ammonium, and a headspace containing 5% ^{12}C- or ^{13}C–CO_2 (v/v). Specifically, microcosms were amended with water (control) or 50 mg NH_4^+-N kg^{-1} dry weight soil (*d.w.s*) per week and incubated at 30% water content (w/w) for 56 days at 28 °C. An additional series of ^{13}C–CO_2 microcosms were amended with 0.01% (v/v) (100 Pa) acetylene (C_2H_2) to inhibit AMO-based ammonia oxidation. All microcosms were prepared in triplicate and destructively sampled. Total DNA was extracted after 56 days incubation and ^{13}C-isotopically enriched DNA was separated in CsCl gradients before fractionation and determination of the distribution of *amoA* genes of comammox, ammonia-oxidizing archaea (AOA) and bacteria (AOB) determined by quantitative PCR. Detailed methods are provided in the Supplemental Information.

Net soil nitrification was determined by measuring increases in NO_3^--N concentrations. After 56 days of incubation, NO_3^- concentrations in forest soil increased by 14.8 and 143 mg NO_3^--N kg^{-1} *d.w.s* in control and NH_4^+-amended microcosms, respectively. In paddy soil, NO_3^- concentrations increased by 68.2 and 405 mg NO_3^--N kg^{-1} *d.w.s* in control and NH_4^+-amended microcosms, respectively (Fig. 1a). Incubation with 0.01% C_2H_2 in the headspace completely inhibited

* Corresponding author.
E-mail address: jiangxj@swu.edu.cn (X. Jiang).

https://doi.org/10.1016/j.soilbio.2019.06.004
Received 4 March 2019; Received in revised form 4 June 2019; Accepted 4 June 2019
Available online 05 June 2019
0038-0717/

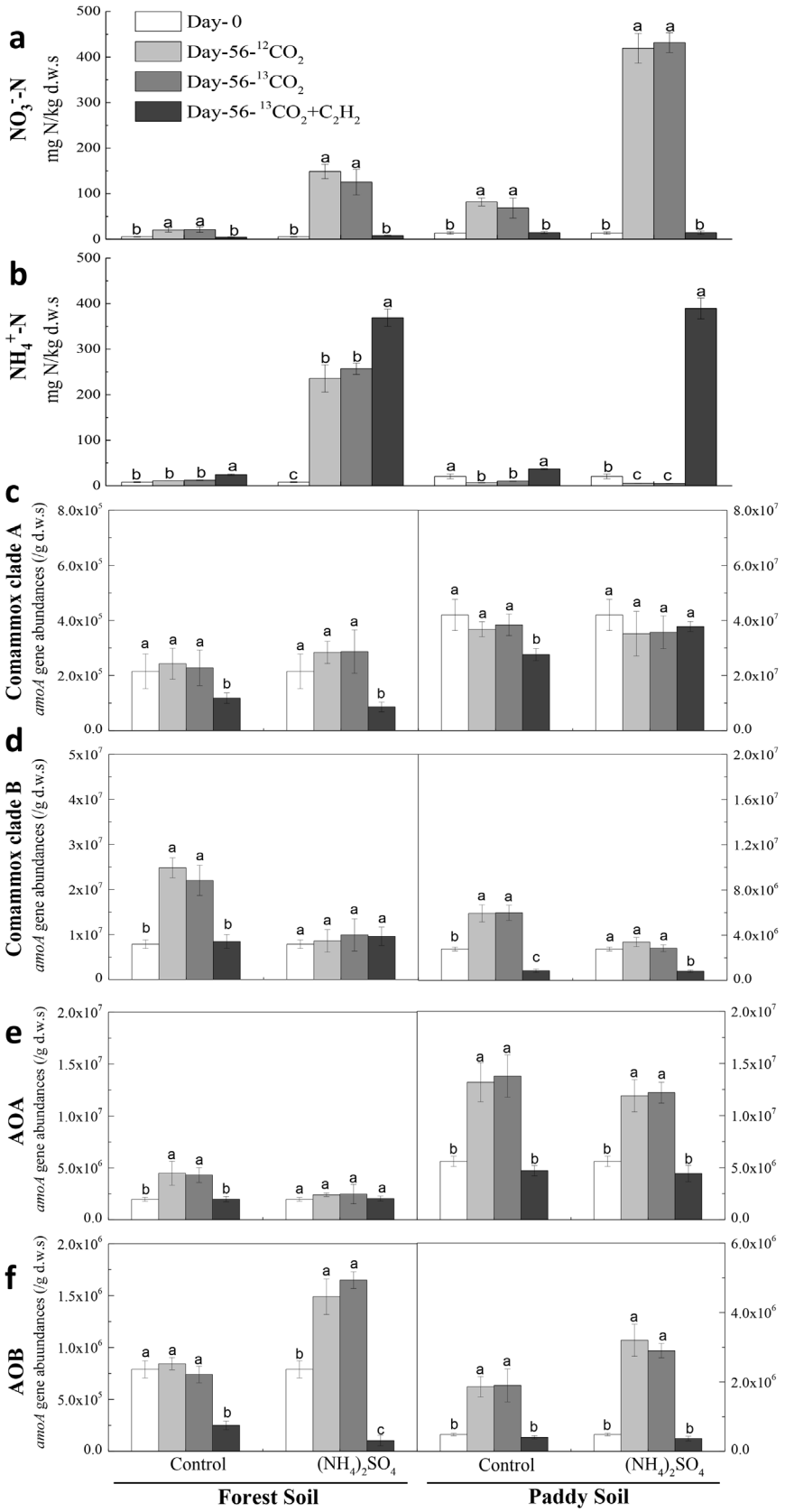

Fig. 1. Changes in concentrations of NO_3^--N (a), NH_4^+-N (b) and *amoA* gene abundance of comammox clade A (c), comammox clade B (d), AOA (e) and AOB (f) in forest and paddy soils incubated with or without $(NH_4)_2SO_4$ (50 mg N/kg/week) application before and after 56 days incubation. There are orders of magnitude differences in abundances of different microbes in different soils, so the Y-axes have different scales both in regard to the same microorganism in different soils, and between the different microorganisms in the same soil. Microcosms contained a headspace of either 5% (v/v) $^{12}CO_2$, 5% $^{13}CO_2$, or 5% $^{13}CO_2$ + 0.1% (v/v) C_2H_2. Error bars represent the standard error of the mean of triplicate microcosms. Different letters above data points indicate a significant difference ($p < 0.05$).

nitrification, demonstrating that AMO-associated autotrophic nitrification was the dominant process (Fig. 1a and b). Quantitative PCR analysis demonstrated that comammox clade B were more abundant than clade A in the forest soil, while clade B abundance was 14 times lower than clade A in the paddy soil (Fig. 1c and d). In both soils, comammox clade B abundance increased significantly ($p < 0.05$) (Fig. 1d) in control microcosms without C_2H_2 and was concomitant with nitrification activity (Fig. 1a), while clade A did not change significantly

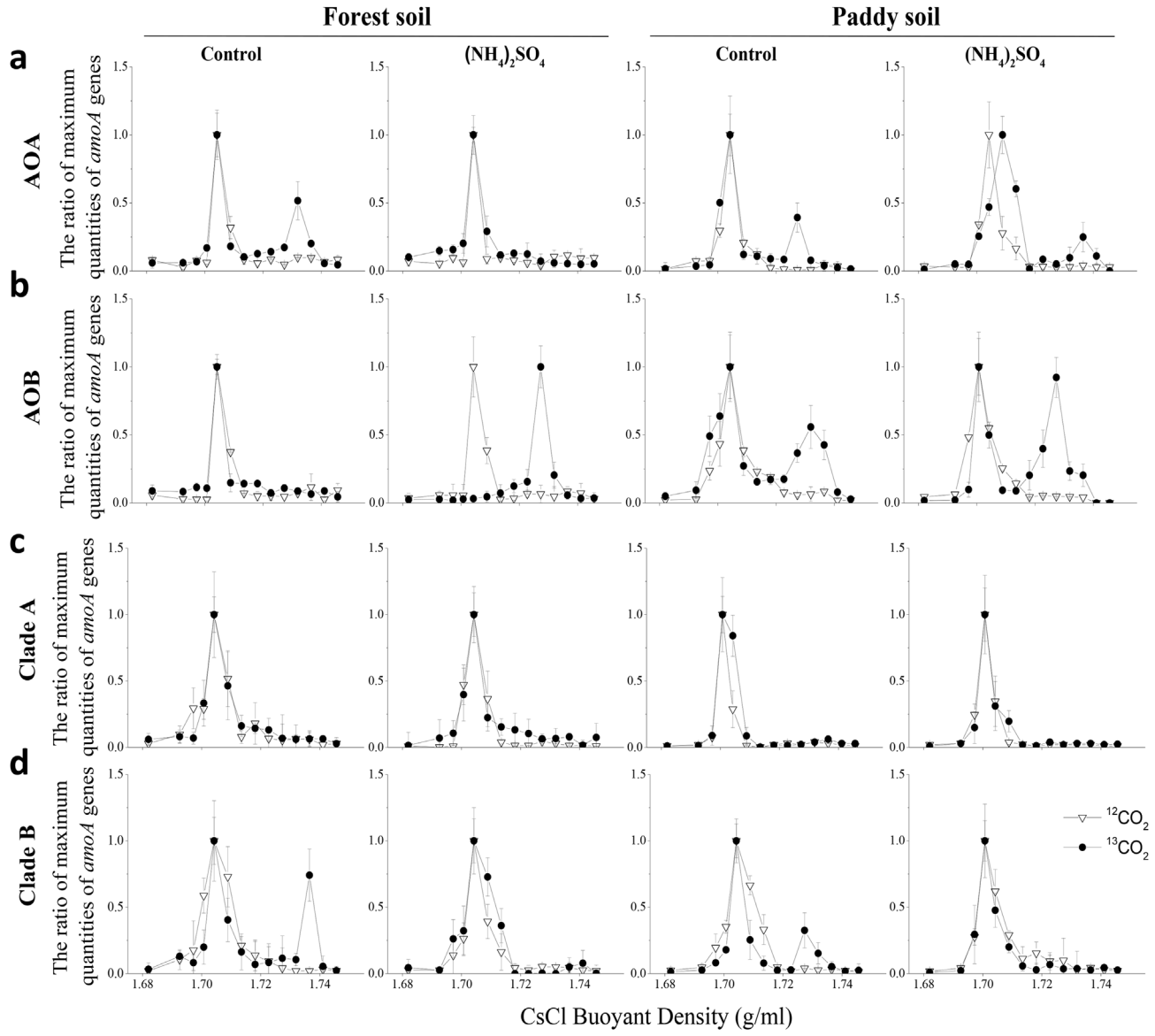

Fig. 2. Distribution of *amoA* genes derived from AOA (a), AOB (b), comammox clade A (c) and comammox clade B (d) in genomic DNA separated in CsCl buoyant density gradients. Forest and paddy soil microcosms were incubated in the presence or absence of $(NH_4)_2SO_4$ with the headspace containing 5% (v/v) $^{12}CO_2$ or $^{13}CO_2$. The normalized data are the ratios of the gene abundance for each DNA gradient to the maximum quantities for each treatment. Error bars represent the standard error of the mean of triplicate microcosms.

($p > 0.05$) during incubation regardless of NH_4^+-amendment or not (Fig. 1c). In the absence of C_2H_2, AOA abundance increased significantly ($p < 0.05$) in all microcosms except for those forest soil microcosms amended with NH_4^+, and AOB abundance increased significantly ($p < 0.05$) in all microcosms except forest soil microcosms containing no NH_4^+ amendment (Fig. 1e and f).

The normalized distribution of *amoA* gene abundances in CsCl gradients derived from $^{12}CO_2$- and $^{13}CO_2$-incubated microcosms was shown in Fig. 2. In forest soil, comparison of the distribution of AOA and AOB *amoA* gene abundances in CsCl gradients derived from $^{12}CO_2$- and $^{13}CO_2$-incubated microcosms demonstrated autotrophic growth of AOA but not AOB without NH_4^+ amendment (Fig. 2a and b). The opposite trend was observed when NH_4^+ was added to forest soil with only AOB genomic DNA enriched in ^{13}C (Fig. 2a and b). In the paddy soil, both AOA and AOB were labeled regardless of ammonium addition (Fig. 2a and b). In both forest and paddy soil unamended microcosms (i.e. no added NH_4^+) incubated with $^{13}CO_2$, comammox clade B *amoA* genes were enriched in genomic DNA with a buoyant density of 1.730–1.735 g ml^{-1}, demonstrating autotrophic growth of comammox clade B (Fig. 2d). However, unlike AOB in both soils, clade B comammox did not grow when inorganic NH_4^+ was added. Incorporation of CO_2-derived C was not observed for comammox clade A under any incubation conditions, with the same distribution of *amoA* genes between ^{12}C and ^{13}C–CO_2 microcosms (Fig. 2c).

Phylogenetic analysis demonstrated that all clade B *amoA* sequences from ^{13}C-enriched growing organisms in paddy soil belonged to a single OTU (> 97% identity) and were closely related to sequence MF347205 obtained from an Italian paddy soil (Fig. S3). Interestingly, the same uncultured clone was found in the forest soil and represented 79% of all sequences. The other 21% were closely related to sequence AJ884548 retrieved from mineral soils (Fig. S3). As for active AOB, *Nitrosospira* 3 and *Nitrosospira* 9 subcluster accounted for 57.8% and 42.6% respectively in the forest soil with NH_4^+ amendment, *Nitrosospira* 3 subcluster accounted for 99.9% in paddy soils with and without NH_4^+ amendment (Fig. S4).The predominant members of active AOA fell into the *Nitrososphaera* cluster, representing 99.4%, 95.8% and 98.4% in forest soil

microcosms without NH_4^+ amendment, paddy soil microcosms with and without NH_4^+ amendment, respectively (Fig. S5), which was expected based on previous SIP results for paddy and forest soils (Wang et al., 2015; Huang et al., 2018). These results indicated a low diversity of active ammonia oxidizers, not only for comammox strains, but also for AOA and AOB — even though more phylotypes for these groups are detected because NGS sequencing was used instead of clone library screening.

These results demonstrate that comammox *Nitrospira* clade B, rather than clade A, contributed to nitrification in two contrasting soils when nitrification was fueled by mineralized organic nitrogen and not from added inorganic NH_4^+. AOA have been previously demonstrated to preferentially utilize ammonium from mineralized organic substrates (He et al., 2012) and low and high rates of ammonium supply in soil have been demonstrated to preferentially select AOA and AOB, respectively (Hink et al., 2018), which indicates that some AOA and comammox clade B populations may utilize the same sources of ammonia in soil. This utilization of ammonia supplied at low concentrations indicates a potential high affinity for ammonia by clade B comammox, as found for cultivated strains of clade A comammox as well as for AOA strains such as *Nitrosopumilus maritimus* (Martens-Habbena et al., 2009), and higher than that of many cultivated AOB strains (Kits et al., 2017). These results therefore provide evidence for comammox (and AOA) to outcompete AOB at low NH_4^+ concentrations in soil. Comammox bacteria did not contribute to nitrification when inorganic ammonium was supplied. As ammonium was unlikely to be a limiting factor due to weekly addition, the lack of growth of comammox bacteria indicates potential inhibition, perhaps by higher ammonia concentrations.

In summary, our results using two contrasting soils demonstrate nitrification activity associated with comammox *Nitrospira* clade B under low N concentrations when ammonia is only supplied from mineralized organic nitrogen, and the lack of comammox growth when exposed to high concentrations of added ammonium indicates that they may not grow under conditions that select for growth of AOB.

Financial interests

The authors declare no competing financial interests.

Acknowledgments

We thank Dr. Holger Daims from the University of Vienna for his valuable suggestions for this study, data interpretation and manuscript preparation. This work was supported by the National Natural Science Foundation of China (41671232), and the National Key Research and Development Program of China (2016YFD0300901). GN is funded by the AXA Research Fund.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.06.004.

References

Bock, E., Wagner, M., 1992. Oxidation of inorganic nitrogen compounds as an energy source. The Prokaryotes 2, 457–495.
Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R.H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P.H., Wagner, M., 2015. Complete nitrification by Nitrospira bacteria. Nature 528, 504–509.
Daims, H., Lücker, S., Wagner, M., 2016. A new perspective on microbes formerly known as nitrite-oxidizing bacteria. Trends in Microbiology 24, 699–712.
He, J., Hu, H., Zhang, L., 2012. Current insights into autotrophic thaumarchaeal ammonia oxidation in acidic soils. Soil Biology and Biochemistry 55, 146–154.
Hink, L., Gubry-Rangin, C., Nicol, G.W., Prosser, J.I., 2018. The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions. The ISME Journal 12, 1084–1093.
Huang, X., Zhao, J., Su, J., Jia, Z., Shi, X., Wright, A.L., Zhu-Barker, X., Jiang, X., 2018. Neutrophilic bacteria are responsible for autotrophic ammonia oxidation in an acidic forest soil. Soil Biology and Biochemistry 119, 83–89.
Kits, K.D., Sedlacek, C.J., Lebedeva, E.V., Han, P., Bulaev, A., Pjevac, P., Daebeler, A., Romano, S., Albertsen, M., Stein, L.Y., Daims, H., Wagner, M., 2017. Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. Nature 549, 269–272.
Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, B.C., Waterbury, J.B., Stahl, D.A., 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437, 543–546.
Kuypers, M.M.M., 2017. A fight for scraps of ammonia. Nature 549, 162–163.
Martens-Habbena, W., Berube, P.M., Urakawa, H., de la Torre, J.R., Stahl, D.A., 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461, 976–979.
Palomo, A., Pedersen, A.G., Fowler, S.J., Dechesne, A., Sicheritz-Pontén, T., Smets, B.F., 2018. Comparative genomics sheds light on niche differentiation and the evolutionary history of comammox Nitrospira. The ISME Journal 12, 1779–1793.
Pinto, A.J., Marcus, D.N., Ijaz, U.Z., Bautista-de lose Santos, Q.M., Dick, G.J., Raskin, L., 2015. Metagenomic evidence for the presence of comammox Nitrospira-like bacteria in a drinking water system. mSphere 1 e00054-15.
Pjevac, P., Schauberger, C., Poghosyan, L., Herbold, C.W., van Kessel, M.A.H.J., Daebeler, A., Steinberger, M., Jetten, M.S.M., Lücker, S., Wagner, M., Daims, H., 2017. AmoA-targeted polymerase chain reaction primers for the specific detection and quantification of comammox *Nitrospira* in the environment. Frontiers in Microbiology 8, 1508.
Santoro, A.E., 2016. The do-it-all nitrifier. Science 351, 342–343.
van Kessel, M.A., Speth, D.R., Albertsen, M., Nielsen, P.H., Op den Camp, H.J.M., Kartal, B., Jetten, M.S.M., Lücker, S., 2015. Complete nitrification by a single microorganism. Nature 528, 555–559.
Wang, B., Zhao, J., Guo, Z., Ma, J., Xu, H., Jia, Z., 2015. Differential contributions of ammonia oxidizers and nitrite oxidizers to nitrification in four paddy soils. The ISME Journal 9, 1062–1075.
Winogradsky, S., 1890. The morphology of the contributions of nitrification system. Archives of Biological Sciences 4, 257–275.