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RESEARCH ARTICLE

# Research trends and hotspots related to ammonia oxidation based on bibliometric analysis

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**Abstract** Ammonia oxidation is the rate-limiting and central step in global biogeochemistry cycle of nitrogen. A bibliometric analysis based on 4314 articles extracted from Science Citation Index Expanded database was carried out to provide insights into publication performances and research trends of ammonia oxidation in the period 1991–2014. These articles were originated from a wide range of 602 journals and 95 Web of Science Categories, among which *Applied and Environmental Microbiology* and Environmental Sciences took the leading position, respectively. Furthermore, co-citation analysis conducted with help of CiteSpace software clearly illustrated that ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), and anaerobic ammonia oxidation (anammox) were three dominant research themes. A total of 15 landmark works identified with the highest co-citation frequencies at every 8 years were extracted, which demonstrated that the establishments of culture-independent molecular biotechnologies as well as the discoveries of anammox and AOA played the most significant roles in promoting the evolution and development of ammonia oxidation research. Finally, word cluster analysis further suggested that microbial abundance and community of AOA and AOB was the most prominent hotspot, with soil and high-throughput sequencing as the most promising ecosystem and molecular biotechnology. In addition, application of anammox in nitrogen removal from wastewater has become another attractive research hotspot. This study provides a basis for better understanding the situations and prospective directions of the research field of ammonia oxidation.

**Keywords** Ammonia-oxidizing archaea · Anammox · Co-citation · CiteSpace · Word cluster analysis

## Introduction

Nitrogen cycle is one of the basic material circulations within the biosphere, including ammonification, nitrification, denitrification, nitrogen fixation, and organic nitrogen synthesis. Ammonia oxidation is the first step of nitrification and also the rate-limiting step and central part of the global biogeochemical cycle of nitrogen (Martens-Habbena et al. 2009). It had long been thought that ammonia oxidation was a chemical reaction with nitrate as the product rather than a biological process by the end of nineteenth century. Winogradsky (1890) firstly found that aerobic ammonia oxidation was catalyzed by a group of chemolithotrophic bacteria with production of nitrite, which was further oxidized to nitrate by another bacterial group. These two kinds of bacteria were further isolated, now known as the ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Over a century, it has been widely believed that only members from bacterial domain were capable of performing ammonia oxidation with

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oxygen as electron donor (Kowalchuk and Stephen 2001). Nevertheless, the research field of ammonia oxidation underwent two revolutionary breakthroughs in the past two decades: the discovery of anaerobic ammonia oxidation (anammox) bacteria and ammonia-oxidizing archaea (AOA) (Mulder et al. 1995; Konneke et al. 2005). Relying on the rapid development of molecular biological technologies in recent years, the phylogenetic and physiological characteristics of anammox and AOA have received rapid and extensive researches, in which the obtained results suggested that they also played a considerable role in ammonia oxidation in a variety of ecosystems (Shen et al. 2014). Therefore, it is essential to illuminate the research hotspots and promising future directions in this field through portraying the already achieved research progresses in order to shed light for the subsequent researches.

Bibliometric, firstly introduced by Pritchard (1969) in English-speaking world, is an effective and useful tool to evaluate the scientific productions and research trends in a specific research field (Li et al. 2014; Lin and Ho 2015). The performances of publication outputs from different countries, institutions, journal, and categories could be obtained through statistical analysis of extracted document information. Research trends are commonly identified by simply analyzing the most frequently used author keywords, which have been used in a variety of research fields, such as tropical medicine (Falagas et al. 2006), volatile organic compounds (Zhang et al. 2010), and engineering nanomaterials (Wang et al. 2014). More recently, word cluster analysis that combines author keywords, *KeyWords Plus*, and substantives in title is proved to be a more effective and comprehensive bibliometric method, which has been successfully applied to reveal the research tendencies and hotspots in the research field of risk assessment (Mao et al. 2010), drinking water (Fu et al. 2013), and pluripotent stem cell (Lin and Ho 2015). Furthermore, co-citation analysis is a powerful method to identify the intellectual structures and research fronts in a research field by analyzing the highly cited references (Appio et al. 2016). Therefore, it is supposed that collaborative applications of co-citation analysis along with word cluster analysis are capable of elucidating not only the research trend but also the roles of landmark works played in the evolution of a research field.

In this study, a bibliometric analysis of ammonia oxidation researches was carried out based on Science Citation Index Expanded (SCI-EXPANDED) database from 1991 to 2014. The documents were analyzed and summarized to quantitatively describe the publication performances including annual outputs, leading countries and institutions, mainstream journals, and Web of Science Categories. More importantly, the intellectual structure, research trends, and hotspots were illustrated in detail to gain insights into the ammonia oxidation research by co-citation analysis and word cluster analysis.

## Methodology

Publication outputs were obtained from the online version of SCI-EXPANDED databases of Web of Science Core Collection on July 18, 2015. "Ammoni* oxid*" were searched in terms of topic including publication title, abstract, author keywords, and *KeyWords Plus* with publication year limitation from 1991 to 2014, as abstract information began to be included in Web of Science in 1991. To be specific, "ammoni* oxid*" included "ammonia oxidation," "ammonia-oxidizing," "ammonia oxidization," "ammonia oxidizer," "ammonium oxidized," "ammonium oxidant," etc. All the specific index words were listed (Table S1). Article was the only considered document type, as it accounted for the majority of publications and represented independent research topics and achievements (Ho et al. 2010). Articles that could only be searched out by *KeyWords Plus* were excluded, because *KeyWords Plus* are index terms created from frequently occurring words in the titles of article's cited references, but in most cases, they were irrelevant to the article topics (Garfield 1990; Fu et al. 2012). Finally, 4314 articles met the selection criteria and used for further analysis.

Downloaded information included authors, affiliations, article title, abstract, author keywords, *KeyWords Plus*, journals, published year, Web of Science categories, and citations in each year for each article. Additional data processing was performed using Microsoft Excel 2010 (Li and Ho 2008). Impact factors in 2014 ($IF_{2014}$) of the journals were obtained from the Journal Citation Reports (JCR) published in 2014. In the single author articles, the author was designated as both first author and corresponding author (Ho 2012). The countries/territories and collaboration types of articles were determined by the addresses of authors. Articles originating from England, Scotland, Northern Ireland, and Wales were reclassified to the UK. Articles from Yugoslavia and Serbia were reclassified to Serbia. Articles from USSR and Russia were taken as being from Russia (Ho 2012). Articles from Hong Kong were assigned to China. The independent or collaborative article was assigned according to the authors' addresses whether from the same country/institute or not (Li and Ho 2008).

Co-citation analysis was conducted by the software CiteSpace 4.0 to identify the intellectual structure and influential works in the research field of ammonia oxidation. Co-citation means that two documents are cited together by one identical article, and frequent co-citations indicate that they share associated research themes (Small 1973). Therefore, co-citation analysis can cluster the related references into groups according to their link strengths. Then, the core themes of the research field can be identified through analysis of articles in each cluster (Pilkington and Meredith 2009). In the CiteSpace, timespan 1991–2014 were divided into 12 time intervals with each slice length of 2 years. The top 50 most cited references from each time interval were extracted to construct the co-citation network. Finally, five landmark works identified with the highest co-citation frequencies in each 8-year stage, i.e., 1991–1998, 1999–2006, and 2007–2014, were further extracted and illustrated.

Word cluster analysis was carried out according to the procedures in previous studies (Mao et al. 2010; Fu et al. 2013). Briefly, author keywords, *KeyWords Plus*, and substantives in titles were extracted from the 4314 articles and combined as the "word source." Next, the frequently appeared synonymic words or phrases were picked up as the "supporting words" and grouped into one "word cluster" as one possible research hotspots with help of the researchers' specialized knowledge. Finally, the flourish or decline of hotspots was revealed represented by the research trends of word clusters by counting the articles containing the corresponding supporting words in their front pages which included titles, abstracts, and author keywords. In addition, co-occurrence of author keywords was also analyzed using the software BibExcel and visualized in Pajek.

## Results and discussion

### Publication outputs

To obtain an overview of the ammonia oxidation research, the annual number of the 4314 articles published in the period 1991–2014 is displayed in Table 1. The annual publication outputs increased steadily from 47 in 1991 to 494 in 2013 but then got a slight decrease to 490 in 2014, demonstrating a tenfold increase over the 24 years. The average co-authors per article almost doubled from 2.8 in 1991 to 5.4 in 2014, with the most 36 authors in one article published in Nature (Strous et al. 2006). The average cited references increased by 86% from 24 in 1991 to 45 in 2014, partially attributing to the more available on-line documents and more convenient access to them. Among the 4314 articles, 4253 articles (99%) were published in English. In addition, the other 61 articles published in other 13 languages were also appeared including Chinese (18), Polish (10), Russian (7), French (5), Japanese (5), German (4), Portuguese (3), Spanish (2), Czech (2), Rumanian (2), and one for Serbo-Croatian, Hungarian, and Malay, respectively. However, all of these non-English articles contained English title, abstract, and author keywords (if they have) in their front page for easy reading.

### Countries/territories and institutions

There were seven articles without author's addresses information in SCI-EXPANDED. The remaining 4307 articles were distributed in 90 countries, in which 3133 (73%) were single country articles from 56 countries and 1742 (27%) were internationally collaborative articles from 84 countries. The top 15 countries which published more than 100 articles are listed in Table 2 with several other collaborative and authored indicators (Chiu and Ho 2005; Ho et al. 2010). The top 15 countries included nine European countries, three Asian countries, two American countries, and one Oceania country, which were dominated by developed countries, demonstrating the positive role of economic capabilities in promoting academic levels. In the BRIC countries, Russia, India, and Brazil published 86 (2.0%), 74 (1.7%), and 48 (1.1%) articles and ranked 16th, 21st, and 23rd, respectively. USA took the leading role that published 964 articles (22%) in total and 366 internationally collaborative articles, demonstrating the strong strength in scientific research and international cooperation, which also revealed in other research areas (Mesdaghinia et al. 2015; Wang et al. 2014). China was the only developing country in the top 15 countries that published a total of 893

**Table 1** Characteristics of the extracted articles by year from 1991 to 2014

| PY | TP | AU | AU/TP | NR | NR/TP | PG | PG/TP |
|---|---|---|---|---|---|---|---|
| 1991 | 47 | 132 | 2.8 | 1127 | 24 | 455 | 9.7 |
| 1992 | 41 | 124 | 3.0 | 991 | 24 | 385 | 9.4 |
| 1993 | 49 | 144 | 2.9 | 1308 | 27 | 426 | 8.7 |
| 1994 | 63 | 189 | 3.0 | 1770 | 28 | 606 | 9.6 |
| 1995 | 56 | 192 | 3.4 | 1664 | 30 | 509 | 9.1 |
| 1996 | 52 | 165 | 3.2 | 1513 | 29 | 457 | 8.8 |
| 1997 | 82 | 311 | 3.8 | 2516 | 31 | 684 | 8.3 |
| 1998 | 90 | 318 | 3.5 | 2397 | 27 | 743 | 8.3 |
| 1999 | 88 | 340 | 3.9 | 2674 | 30 | 800 | 9.1 |
| 2000 | 93 | 340 | 3.7 | 2599 | 28 | 760 | 8.2 |
| 2001 | 106 | 387 | 3.7 | 3290 | 31 | 955 | 9.0 |
| 2002 | 120 | 436 | 3.6 | 3475 | 29 | 982 | 8.2 |
| 2003 | 135 | 583 | 4.3 | 4318 | 32 | 1250 | 9.3 |
| 2004 | 155 | 673 | 4.3 | 4564 | 29 | 1290 | 8.3 |
| 2005 | 175 | 761 | 4.3 | 5839 | 33 | 1573 | 9.0 |
| 2006 | 155 | 720 | 4.6 | 5334 | 34 | 1372 | 8.9 |
| 2007 | 216 | 992 | 4.6 | 7433 | 34 | 1962 | 9.1 |
| 2008 | 241 | 1064 | 4.4 | 9321 | 39 | 2233 | 9.3 |
| 2009 | 284 | 1332 | 4.7 | 10,336 | 36 | 2431 | 8.6 |
| 2010 | 310 | 1547 | 5.0 | 12,469 | 40 | 2739 | 8.8 |
| 2011 | 361 | 1822 | 5.0 | 15,268 | 42 | 3198 | 8.9 |
| 2012 | 411 | 2103 | 5.1 | 17,306 | 42 | 3795 | 9.2 |
| 2013 | 494 | 2613 | 5.3 | 22,537 | 46 | 4858 | 9.8 |
| 2014 | 490 | 2628 | 5.4 | 21,808 | 45 | 4781 | 9.8 |

*TP* total articles, *AU* author numbers, *AU/TP* average author numbers per article, *NR* cited reference numbers, *NR/TP* average cited reference numbers per article, *PG* page numbers, *PG/TP* average page numbers per article

**Table 2** Publication performances of the top 15 most productive countries with TP > 100

| Country/territory | TP | TP R (%) | IP R (%) | CP R (%) | FP R (%) | RP R (%) | SP R (%) |
|---|---|---|---|---|---|---|---|
| USA | 964 | 1 (22) | 2 (19) | 1 (31) | 2 (17) | 2 (16) | 1 (16) |
| China | 893 | 2 (21) | 1 (20) | 2 (24) | 1 (19) | 1 (19) | 11 (1.8) |
| Germany | 422 | 3 (10) | 4 (5.1) | 3 (22) | 5 (5.9) | 5 (5.9) | 4 (8.1) |
| Japan | 421 | 4 (10) | 3 (10) | 6 (10) | 3 (8.0) | 3 (8.1) | 5 (7.2) |
| Netherlands | 382 | 5 (8.9) | 5 (4.9) | 4 (20) | 4 (6.1) | 4 (6.0) | 7 (5.4) |
| UK | 260 | 6 (6.0) | 6 (3.8) | 5 (12) | 6 (4.2) | 6 (4.1) | 8 (3.6) |
| Spain | 199 | 7 (4.6) | 7 (3.3) | 8 (8.3) | 7 (3.5) | 7 (3.5) | N/A |
| Australia | 164 | 8 (3.8) | 12 (2) | 7 (8.6) | 9 (2.5) | 9 (2.4) | 16 (0.9) |
| France | 155 | 9 (3.6) | 16 (1.9) | 9 (8.2) | 10 (2.2) | 10 (2.2) | N/A |
| South Korea | 147 | 10 (3.4) | 8 (2.6) | 12 (5.5) | 8 (2.9) | 8 (3.0) | 11 (1.8) |
| Canada | 141 | 11 (3.3) | 9 (2.3) | 11 (6.0) | 11 (2.1) | 11 (2.1) | 10 (2.7) |
| Denmark | 141 | 11 (3.3) | 17 (1.9) | 10 (7.1) | 12 (2.0) | 12 (2.1) | 11 (1.8) |
| Italy | 103 | 13 (2.4) | 12 (2.0) | 17 (3.4) | 13 (2.0) | 13 (1.9) | 16 (0.90) |
| Belgium | 102 | 14 (2.4) | 19 (1.5) | 14 (4.7) | 15 (1.8) | 15 (1.8) | N/A |
| Sweden | 102 | 14 (2.4) | 18 (1.6) | 15 (4.3) | 14 (1.8) | 14 (1.9) | N/A |

*TP* total number of articles, *TP R* (%) rank and the percentage of total articles, *IP R* (%) rank and the percentage of independent articles, *CP R* (%) rank and the percentage of international collaborative articles, *FP R* (%) rank and the percentage of first authored articles, *RP R* (%) rank and the percentage of the corresponding authored articles, *SP R* rank and the percentage of the single authored articles, N/A not available

articles (21%) and ranked second to USA. However, China presented a strong growth momentum during the past decade that the annual article number increased from 12 in 2006 to 211 in 2014 and surpassed USA in 2009 (Fig. S1). On one hand, this rapid growth could be attributed to the steadily increasing investment in basic research by Chinese government during the past two decades (NBS and MST 2015). On the other hand, the abruptly accelerated growth of annual publications from China since 2006 was greatly contributed by a groundbreaking academic breakthrough, i.e., the discovery of AOA, which triggered vast follow-up researches. This will be discussed in detail later.

At the institution level, the 4307 articles available with affiliations came from 2480 institutes in 90 countries, in which 1801 (42% of 4307 articles) were single institute articles and 2506 (58%) were inter-institutionally collaborative articles, including 1174 internationally collaborative articles and 1332 nationally collaborative articles. Five institutes in China, two in Netherlands, and one each in USA, UK, Germany, Australia, Russia, Austria, Belgium, and Japan were ranked in the top 15 most productive institutes (Table S2). Chinese Academy of Sciences took the leading position not only in total publications (236, 5.5%) but also in independent articles (52, 2.9%), internationally collaborative articles (89, 7.6%), and nationally collaborative articles (95, 7.1%). Moreover, articles published by Chinese Academy of Sciences exhibited an accelerated growth rate since 2006, in consistent with the trend of China (Fig. S2). However, there is a bias for Chinese Academy of Sciences that covers over 100 branches all over the country, and articles from all these branches were pooled together in this study (Li et al. 2011). It was notably that articles from Harbin Institute of Technology from China showed a rapid growth after 2011 and became the second place only 2 years later, demonstrating the rapid development in this present research field. In addition, it was notable to see that higher than 60% of articles were generally collaborated by two or more institutes except for Oregon State University (41%) and Russian Academy of Sciences (38%). The percentage of collaborative articles from the Radboud University Nijmegen (Netherlands) and University of Vienna (Austria) even reached up to 93% of 80 articles and 98% of 48 articles, respectively. It could be inferred that the collaboration is becoming a popular trend as the enhancement of cooperative communication between different institutes and countries.

## Web of science categories and journals

The investigation of subject categories and journals was an effective way to map the research field and direction of a research topic (Chuang and Ho 2014). The present 4314 articles were distributed in 95 Web of Science Categories. "Environmental Sciences" contributed the most with 1133 articles (26%), followed by "Microbiology" (1002; 23%) and "Biotechnology and Applied Microbiology" (967; 22%) (Fig. 1). According to the category description in Web of Science, the three categories include resources concerning the study of the environment, microorganisms, and manipulation of living organisms, respectively. As the

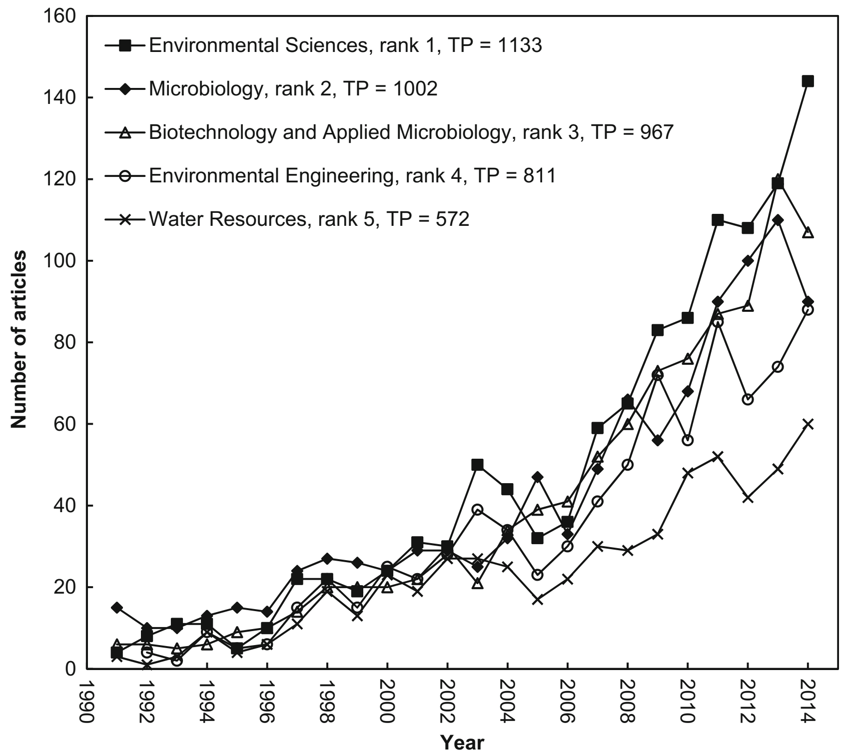


**Fig. 1** Growth trends of annual publications from the top five productive categories

central link of nitrogen biogeochemical cycle and its dominant driver, ammonia oxidation and ammonia-oxidizing microorganisms are two significant aspects of environmental and microbiology research, respectively. It could be concluded that the three most productive categories are unlikely to be exceeded by other categories in the future.

The 4314 articles were published in a wide range of 602 journals, among which the 13 most productive journals ($TP > 50$) published a total of 1198 articles (37%). *Applied and Environmental Microbiology* published the most articles (248, 5.7%), followed by *Water Science and Technology* (243, 5.6%), and *Water Research* (200, 4.6%), which demonstrated that there was not a journal taking the dominant position (Table S3). Moreover, there were still 515 (86%) journals contained less than 10 articles and 281 journals (55%) contained only one article. This phenomenon indicated the broad interests in ammonia oxidation from various research angles, which also appeared in research field of, for instance, homeopathy (Chiu and Ho 2005), risk assessment (Mao et al. 2010), and drinking water (Fu et al. 2013). In terms of the annual article counts, the three most productive journals presented big fluctuations during the past two decades instead of a significantly rising trend, whereas the articles in *Bioresource Technology* showed an intriguingly exponential growth since 2006 and increased to the annually most productive journal after 2011 (Fig. S3), indicating the shift of the journal aims to the ammonia oxidation research.

## Research themes and landmark works by co-citation analysis

To elucidate the intellectual base of the downloaded documents and to capture the influential works in the research field of ammonia oxidation, co-citation analysis was conducted using the software CiteSpace. Finally, a total of 77,879 valid references were extracted and a network consisting of 269 nodes and 900 edges was visualized. The biggest linked clusters containing 161 nodes were illustrated in cluster view (Fig. 2). The tree-ring nodes with tone variations from cool to warm represent the individual cited reference. The warmer the color, the closer the time is. For an individual node, the thickness of each heterochromatic concentric ring represents the co-citation frequencies of the document in the corresponding 2-year time slice. Therefore, the size of each node is proportional to the total co-citation frequencies of the corresponding documents. The top five biggest tree-rings in each 8-year stage were marked with black dots in the center, which were supposed to be the landmark works in the evolution of ammonia oxidation research that achieved groundbreaking discoveries and imposed profound influences on the follow-up researches in this area. The links of the retrieved documents were expressed by connecting lines that the color and distance of the connecting line between two nodes denote the first co-citation year and correlation strength of the corresponding two publications.

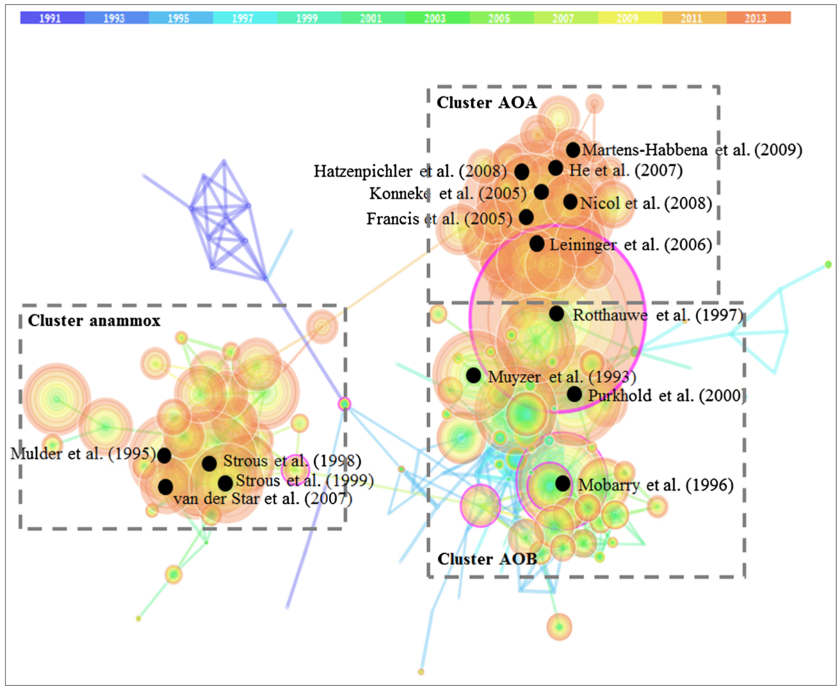


**Fig. 2** Cluster view of the co-citation analysis by CiteSpace

Obviously, the retrieved documents were clustered into three groups, indicating that there were three research themes in the research field of ammonia oxidation (Fig. 2). The theme of each cluster was extracted from the title and keywords of the inclusive articles. The largest cluster consists of 74 members with research theme labeled with ammonia oxidizing bacteria, including highly cited articles Rotthauwe et al. (1997), Mobarry et al. (1996), Purkhold et al. (2000), and Muyzer et al. (1993). Cluster 2 was composed of 34 compact nodes with topic of ammonia oxidizing archaea, while cluster 3 has 32 anammox-related documents. This result demonstrated that AOB, AOA, and anammox composed the intellectual structure of the ammonia oxidation research. Through analysis of the members in each cluster, it was found that the mean publication year of documents in cluster 1 was 1994 with the most recent one published in 2003. In contrast, the other two research topics AOA and anammox were formed in more recent times, with the mean publication year of 2007 and 1999, respectively.

To shed light on the influences of landmark works on the subsequent researches, the top nine articles with the highest annual co-citation frequencies in the whole survey time were illustrated in detail (Fig. 3). Inspecting their research contents, it is easy to conclude that the four AOB-related articles, Rotthauwe et al. (1997), Mobarry et al. (1996), Purkhold et al. (2000), and Muyzer et al. (1993), achieved high co-citation frequencies attributed to *amoA*-targeted DNA primer, 16S rRNA-targeted DNA probe, AOB phylogeny with comparative 16S rRNA and *amoA* sequences, and DGGE analysis method, respectively. These studies provided effective and powerful molecular methods and tools to evaluate the diversity and abundance of ammonia oxidizing bacterial populations in natural and engineered ecosystems. It is interesting to see that the citation trend of Rotthauwe et al. (1997) got an abrupt accelerated growth since 2006 after 10 years steady growth, while the citations of the other three articles tended to be stable or slightly decreasing in recent years since 2008. Therefore, it can be concluded in AOB researches that *amoA*-targeted DNA primer became a more popular tool, whereas 16S rRNA probe or DGGE analysis was no longer mainstream employed methods. For AOA-related researches, Francis et al. (2005), Leininger et al. (2006), and Konneke et al. (2005) were the three most co-cited articles, which firstly revealed the predominance of AOA in ocean, in soil, and isolation of the first AOA strain, respectively. Moreover, Francis et al. (2005) designed the first DNA primer targeting archaeal *amoA* gene, which made the greatest contribution to its second highest co-citation frequency achieved within only 10 years. It can be seen that the three AOA articles received immediate and intensive attentions after their publications and continued to recent years (Fig. 3), demonstrating that the discovery of AOA created an attractive and significant research topic which became the hotspot in ammonia oxidation research. More interestingly, the growth trend of Rotthauwe et al. (1997) after 2006 significantly coincides with those of the three AOA-related articles, indicating that the former abrupt accelerated growth is probably driven by the latter three AOA-related articles. Moreover, the co-citation counts of Rotthauwe et al. (1997) with Francis et al. (2005), Leininger et al. (2006), and Konneke et al. (2005) were 293, 253, and 223, respectively. The values were comparable with the highest co-citation count 335 achieved

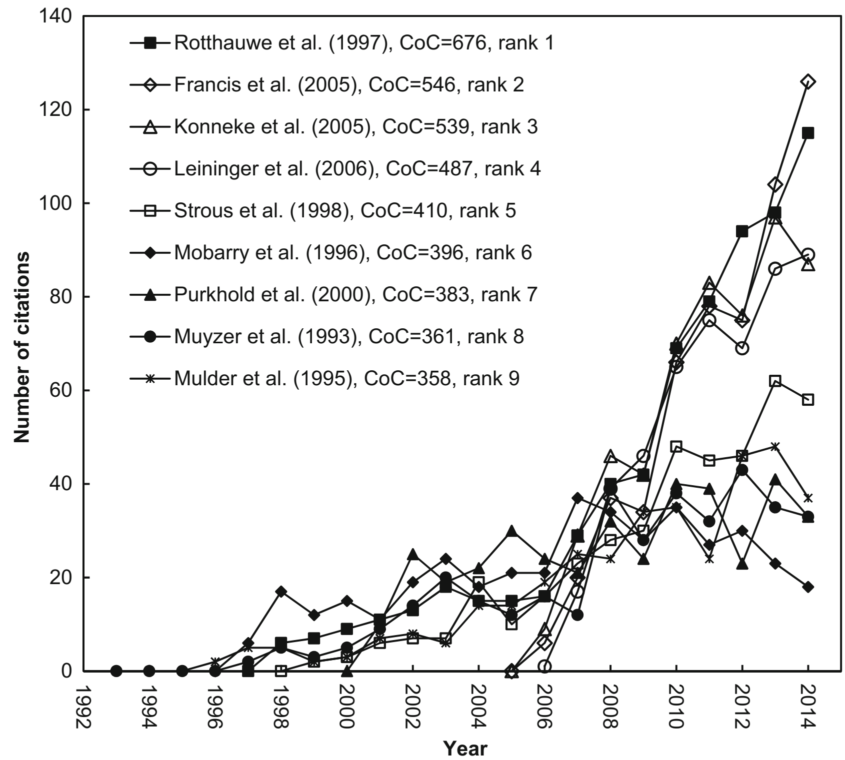


**Fig. 3** Growth trends of annual co-citation frequencies of the top nine landmark works

between two AOA articles, Francis et al. (2005) and Konneke et al. (2005), which demonstrated that the research findings in the article Rotthauwe et al. (1997) played significant roles in the subsequent AOA researches. Considering the intellectual relationship, it could be further inferred that AOB were comparatively studied in quite a number of AOA researches. The direct evidences will be given in the following sections. In anammox researches, the two most co-citation articles were Strous et al. (1998) and Mulder et al. (1995), which provide a powerful anammox enrichment technique and firstly discovered anammox, respectively. These two landmark works triggered great interests in the following anammox-related researches, reflected by their steadily increasing citations during the past two decades.

To further exhibit the intellectual evolution of the ammonia oxidation research, the co-citation result was further depicted in Timezone view, in which the retrieved publications were arranged chronologically according to its first co-citation year (Fig. 4). It is interesting to see that there are two flourishing periods in the ammonia oxidation research throughout the entire timespan, i.e., the second half of 1990s and 2000s. Moreover, these articles concentrated in this two periods are still active in influencing the most recent ammonia oxidation research, reflected by the bright red outer ring of the nods. It was notable that four out of the five landmark works (Martens-Habbena et al. 2009; He et al. 2007; Nicol et al. 2008; Hatzenpichler et al. 2008) in the recent 8-year stage (2007–2014) focused on AOA-related researches, including kinetics, diversity, abundance, and new isolate of AOA. The latest highly co-cited article (Pester et al. 2012) also interested in the investigation of AOA phylogenetic characteristics. These results clearly demonstrated that AOA-related researches represented the current research hotspots in the field of ammonia oxidation.

## Research trends and hotspots by word cluster analysis

Word cluster analysis has been proved to be a more effective and comprehensive tool than author keywords analysis to reveal the research trends and discovering hotspots in a given research field (Mao et al. 2010; Fu et al. 2013). First of all, the three most prominent word clusters pertaining to AOB, AOA, and anammox were extracted contained 2829 articles accounting for as high as 67% of the total 4314 articles (Fig. 5a), which was congruent with the three dominant themes revealed by co-citation analysis. In addition, the other prominent research hotspots were classified into three categories, phylogenetic investigations (Fig. 5b), molecular biotechnologies (Fig. 5c), and ecological environments (Fig. 5d). Moreover, the co-occurrence frequencies of different word clusters were statistically analyzed and listed in the matrix (Table 3).

Word cluster AOB contained the most 1820 articles, and its annual articles always took the leading role during the time period 1991–2014 (Fig. 5a). Moreover, the growth of article numbers obviously accelerated after 2006 and

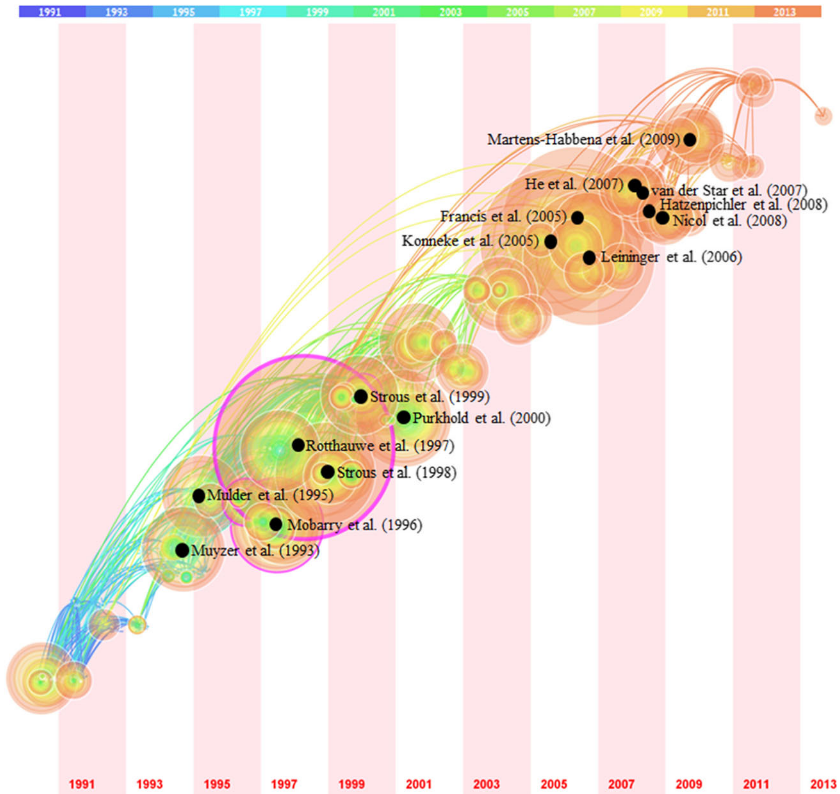


**Fig. 4** TimeZone view of co-citation analysis by CiteSpace

interestingly accompanied the sharp increase of word cluster AOA. It could be further concluded that the exceeding publications significantly rely on the external impetus generated by the accompanied AOB studies in AOA-related researches, as AOB were referred to 407 AOA articles in their front page among the total 699 articles in cluster AOA (Table 3), which could also be referred by the keywords co-occurrence analysis (Fig. S4). This result is echoed by the co-citation results, i.e., the accelerated co-citation trend of Rotthauwe et al. (1997) and its coincident co-citation trend with the three most highly co-cited AOA articles. With regard to AOA, its discovery challenged the long-existed traditional view that bacteria were the only microbial population mediating the ammonia oxidation process; therefore, strong interests were triggered in studying their physiologies and phylogenetic affiliations and the role they played in different ecosystems, reflected by the almost linear growth of cluster AOA since its discovery. Word cluster anammox contained a total of 841 articles which got a steady growth since 2000. Unlike AOA, anammox received little attentions during the first several years after its discovery, which was probably due to its notoriously long generation period and insufficient molecular biotechnology in 1990s (Kartal et al. 2013). The phenomenon of anammox was firstly discovered from the denitrifying fluidized bed reactor, in which ammonia was surprisingly disappeared in proportional to nitrate (Mulder et al. 1995). It was further testified that anammox bacteria use nitrite as electron acceptor to consume ammonia and produces dinitrogen gas as the end product under anaerobic conditions (van de Graaf et al. 1997; Kuenen 2008). The discovery of anammox overturned the traditional knowledge that oxidation of ammonia could only proceed under aerobic condition. With the first successful enrichment of anammox bacteria and development of molecular biotechnology (Strous et al. 1999), anammox-related research received increasing attentions and made rapid progress.

Microbial abundance and community-related articles increased rapidly in the past two decades in the ammonia oxidation research field (Fig. 5b). The two topics respectively answered two fundamental and significant questions, i.e., how many microbial populations existed and who they were (Gao et al. 2013; Niu et al. 2016). There were 681 articles that simultaneously involved these two topics among the 1093 abundance-related and 1530 community-related articles, indicating that they were usually investigated concomitantly (Table 3). As a high majority of microorganisms (99%) are unculturable in lab conditions, microbial phylogenetic analysis largely relies on culture-independent technologies (Pham and Kim 2012). As a consequence, although pure AOB isolates were isolated as early as 1890s, studies on microbial abundance and diversity in various ecosystems really began and took off only after the molecular biomarker and

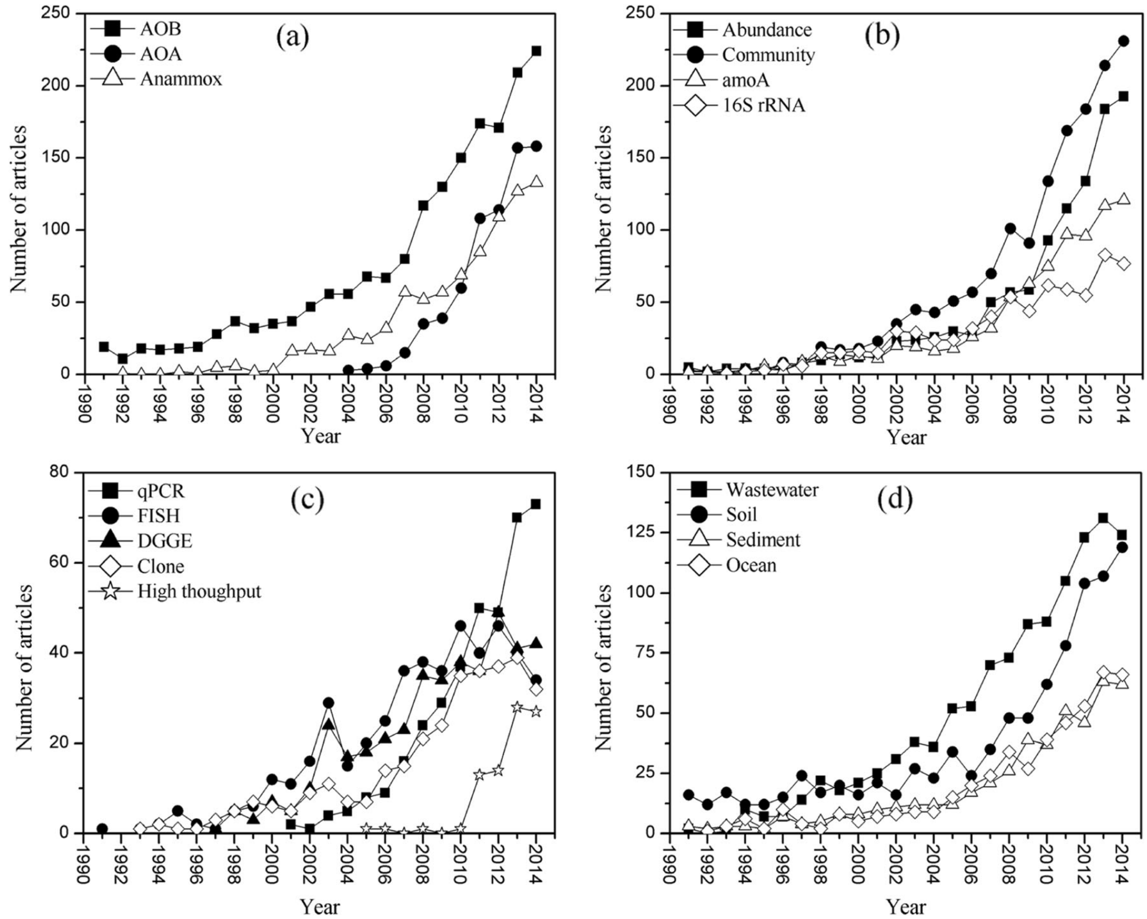


**Fig. 5** Growth trends of articles related to hotspots of ammonia-oxidizing microorganisms (**a**), phylogenetic investigations (**b**), molecular biotechnologies (**c**), and ecological environments (**d**) revealed by word cluster analysis

**Table 3** Co-occurrence frequencies of hotspots-related word clusters

| | AOB | AOA | Anammox | Abundance | Community | amoA | 16S rRNA | Wastewater | Soil | Sediment | Ocean | Nitrogen removal | Partial nitrification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AOB | 1820 | **407** [a] | 78 | 640 | 971 | 616 | 406 | **567** | 541 | 188 | 150 | 204 | 186 |
| AOA | | 699 | 61 | **456** | **532** | **444** | 196 | 55 | **332** | 163 | 200 | 24 | 13 |
| Anammox | | | 841 | 188 | 281 | 57 | 170 | **345** | 10 | 153 | 148 | **361** | **217** |
| Abundance | | | | 1093 | **681** | 465 | 289 | 221 | 386 | 206 | 190 | 115 | 59 |
| Community | | | | | 1530 | 572 | 567 | 393 | 524 | 276 | 249 | 173 | 97 |
| amoA | | | | | | 827 | 269 | 136 | 359 | 144 | 156 | 43 | 25 |
| 16S rRNA | | | | | | | 693 | 186 | 190 | 134 | 145 | 79 | 39 |
| Wastewater | | | | | | | | 1138 | 78 | 51 | 29 | **381** | **246** |
| Soil | | | | | | | | | 907 | 75 | 63 | 12 | 13 |
| Sediment | | | | | | | | | | 464 | 182 | 35 | 13 |
| Ocean | | | | | | | | | | | 465 | 20 | 4 |
| Nitrogen removal | | | | | | | | | | | | 615 | **206** |
| Partial nitrification | | | | | | | | | | | | | 405 |

[a] The numbers in bold indicate that they were mentioned in the text

biotechnologies became available and popular in the past two decades. It was notable that the article counts dealing with abundance and microbial community reached 456 and 532 among the 699 AOA-related articles, demonstrating that they were the dominant research hotspots in AOA researches. This was consistent with that "Ecological communities of ammonia-oxidizing archaea and bacteria" ranked in the top 10 research fronts of 2014 in research area "Ecology and Environmental Sciences" (Thomson Reuters 2014). Due to the high conservativeness and specificity, 16S rRNA gene was the most efficient biomarker to identify the phylogenetic affiliations of bacterial and archaeal species (Yang et al. 2016). The classification of ammonia-oxidizing bacteria through sequencing 16s rRNA genes was firstly investigated by Head et al. (1993). So far, AOB were recognized belonging to three genuses in two lineages, *Nitrosomonas* and *Nitrosospira* from *β-proteobacteria* and *Nitrosococcus* from *γ-proteobacteria* (Purkhold et al. 2000; Monteiro et al. 2014). All the known anammox bacteria distributed in six Candidatus genera of *Planctomycetes* phylum (Ibrahim et al. 2016). AOA were firstly identified as a member of *Crenarchaea* after its discovery but further assigned to a new archaeal phylum named *Thaumarchaea* based on the analysis of the small subunit (SSU) and large subunit (LSU) rDNA gene sequence, archaeal ribosomal proteins, Clusters of Orthologous Groups of proteins (COGs) and comparative genomics (Brochier-Armanet et al. 2008). Besides, *amoA* gene that encodes the unique ammonia monooxygenase α-subunit associated with the function of aerobic ammonia oxidation has surpassed 16S rRNA in 2009 and become the most frequently used biomarker for detecting AOB and AOA in various ecosystems (Fig. 5b). It is precisely because Rotthauwe et al. (1997) and Francis et al. (2005) exploited the specific gene primer targeting bacterial and archaeal *amoA* gene that they take the leading position in the AOB cluster and AOA cluster as mentioned in the co-citation analysis. Therefore, the accompanied investigation of abundance and microbial community of ammonia-oxidizing archaea and bacteria with help of their functional gene maker *amoA* is a prominent hotspot in the research field of ammonia oxidation.

Culture-independent molecular biotechnologies highly boost the development of microbial investigation (Fig. 5c). Fluorescent in situ hybridization (FISH) is a simple and rapid molecular technique to determine the microbial abundance and community structure (Third et al. 2001). However, researches employing FISH technique did not continue the increase trend since 2008 onwards (Fig. 5c) due to its insensitivity and inefficiency when applied in environmental samples with low numbers of microbial cells. In contrast, polymerase chain reaction (PCR), which could realize exponential amplification of a certain fragment of the target genes with the restriction of specific primer, provided a sensitive method and become the mainstream technique to determine microbial population in complex ecosystems (Junier et al. 2010). To be specific, the abundance could be obtained through quantitative PCR (qPCR) analysis, and community structure could be illuminated by analyzing the distinct sequences of PCR-amplified products including denaturing gradient gel electrophoresis (DGGE), clone library, and high-throughput sequences. Although DGGE and clone library are two widely employed and effective molecular technique to explore microbial compositions (Nicolaisen and Ramsing 2002; Quan et al. 2008), they are gradually being replaced by high-throughput sequencing technology, which has demonstrated powerful advantages in deciphering microbial abundances and compositions of achieving tens of thousands of reads at one time with low cost and simple operation (Shen et al. 2014; Zheng et al. 2015).

Ocean, soil, sediment, and wastewater treatment plants are significant ecosystems for global nitrogen cycle, and also the active habitats of ammonia oxidizing microorganisms (Fig. 5d). It has been generally accepted that AOB hold dominant position in the various natural and engineered ecosystems before. However, the discovery of anammox and AOA raised great curiosity concerning their distributions and applications in these systems. Denitrification (bacterial reduction of nitrate to $N_2$) has been believed to be the only significant process for nitrogen sink in marine ecosystems before discovery of anammox. Now, it has been recognized that anammox are active in oceanic oxygen-minimum zones and may contribute up to 50% of nitrogen loss in the ocean (Kuenen 2008). Moreover, AOA has been identified to be dominant over AOB in the oceanic oxygen-minimum zones and suboxic seawater columns (Francis et al. 2005; Santoro et al. 2011) as archaeal AMO had higher affinities to oxygen than AOB (Martens-Habbena et al. 2009). The apparent preference of AOA led to the consideration that AOA might be coupled to anammox that provide 30–40% nitrite required by anammox in the OMZ of the Black Sea and off Peru (Lam et al. 2007; Lam and Kuypers 2011). Thus, the researches referred to marine or ocean have represented a steady increase trend since the discovery of anammox and AOA (Fig. 5d). Since the first investigation of AOA in soils by Leininger et al. (2006), more researches have assessed their abundances and diversities in soils, as shown by the accelerated rising trend of soil cluster. It has been suggested that AOA are numerically and functionally dominant over AOB in a large proportion of investigated soils, especially in acid soils due to the niche preference of AOA for low pH (He et al. 2012). It is notable to see that the most preferred ecosystem for AOA related articles is soil, followed by ocean and only a few dealing with wastewater. As we know, the high concentration of ammonia in wastewaters is a potential hazard to the natural environment and the conventional biological nitrogen removal relies on the autotrophic nitrification mediated by AOB and NOB and subsequent heterotrophic denitrification. After the discovery of anammox, it was realized that the special bacteria had great potential for the removal of undesired ammonia from wastewater, as this process would be much cost-effective than conventional system due to no requirement of aeration and organic carbon as well as lower alkalinity consumption and sludge production (Kuenen 2008). As a result, the prominent potential triggered great research interests in developing the anammox process for nitrogen removal from wastewater. It could be seen that a large amount of anammox articles were related to wastewater, nitrogen removal, and partial nitrification (Table 3 and Fig. S4), suggesting that anammox process for nitrogen removal from wastewater is another prominent research hotspot in the research field of ammonia oxidation. So far, several successful configurations combining anammox and partial nitrification have been proposed, such as SHARON (single reactor system for high rate ammonium removal over nitrite)-anammox, CANON (completely autotrophic nitrogen removal over nitrite), and OLAND (oxygen-limited autotrophic nitrification-denitrification) (Bagchi et al. 2012). It is believed that anammox will be employed by more wastewater treatment plants to remediate nitrogen contamination in the future.

## Conclusions

In this bibliometric study, publication performances and research trends of ammonia oxidation were evaluated by combining co-citation analysis and word cluster analysis. A total of 4314 articles were extracted from SCI-EXPANDED databases during the period 1991–2014. The annual publication output had increased by about ten times over the 24 years. USA was the most productive country with 964 articles, whereas China presented a strong growth momentum during the past decade and was expected to surpass USA in 2 years. *Applied and Environmental Microbiology* and Environmental science took the leading position among the 602 Journals and 95 Web of Science Categories. Co-citation analysis illustrated that AOB, AOA, and anammox constituted the intellectual base of ammonia oxidation research. Highly co-cited articles played a significant role in promoting the evolution and development of the research owing to the effective methods or groundbreaking discoveries they achieved. Word cluster analysis revealed that the abundance and microbial community of ammonia-oxidizing archaea and bacteria as well as application of anammox process for nitrogen removal from wastewater are two most prominent hotspots in the research field of ammonia oxidation.

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