



Does the source of carbon influence the abundance of *nirK*, *nirS* and *nosZ* functional genes in laboratory denitrification bioreactors?

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Biological denitrification in soil is the main producer of nitrous oxide (N_2O) emissions. Denitrifying soil microbes are capable of reducing nitrate (NO_3^-) to nitrite (NO_2^-) to N_2O and di-nitrogen gas (N_2). One third of these denitrifiers possess a truncated functional gene pathway, which may lack the *nosZ* gene and emit N_2O as a final emission product instead of the more benign N_2 . A carbon rich environment, specific to certain types of carbon sources, has been shown to foster an anaerobic environment, which positively impacts microbial denitrification rates. The present study examined the effect of varying carbon sources in laboratory-scale denitrification bioreactors on NO_3^- removal and also correlated performance with the abundance of the denitrifying microbial consortia possessing the denitrifying functional genes *nirK*, *nirS* and *nosZ* in each bioreactor. The bioreactors comprised either lodgepole pine woodchips (LPW), lodgepole pine needles (LPN), barley straw (BBS), or cardboard (CCB), each mixed with soil in a 1:1 ratio (by volume) and subject to sequentially increasing hydraulic loading rates of 3, 5 and 10 $cm\ d^{-1}$ for a total operation period of up to 744 days. A reactor containing soil only (CSO) was used as the study control. The abundance of denitrifiers was determined by targeting *nirK*, *nirS*, *nosZ* functional genes and the overall microbial population was determined by targeting bacterial and archaeal 16sRNA genes. Nitrate removal from all bioreactors was $> 99.7\%$, but when pollution swapping was considered, this ranged from 67% for LPW to 95% for the CCB; this was also mirrored in the average *nirK/nirS/nosZ* gene abundance (CCB, c. 94% (c. 10^8); LPN, 75% (c. 10^7); BBS, c. 74% (c. $10^6/10^7$); LPW, 70% (c. 10^5). Bacterial 16sRNA gene abundance was similar in all reactors including the control ($P=0.0362$). The abundance of *nosZ* genes and the genetic potential for N_2 emissions varied in all reactors in comparison to the control CSO, BBS ($P=0.0051$); CCB ($P=0.0171$); LPN ($P= 0.0049$) and LPW ($P= 0.0008$). Interestingly, *nirS* gene abundance was on average much higher than that of *nirK* and *nosZ* in the LPN and LPW reactors compared to the CSO, BBS and CCB reactors, indicating a habitat/carbon source preference for denitrifying organisms. Indeed, the high abundance of *nir* genes in comparison to the total bacterial abundance indicates the possible denitrifying role of fungal and archaeal organisms, which warrants further investigation. The addition of carbon had a direct impact on denitrifier abundance (CSO- $10^4/10^5$; CCB/BBS/LPN/LPW- $10^7/10^8$).