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Commentary

How to disentangle microbially functional complexity: an insight from the network analysis of C, N, P and S cycling genes

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A complete ecosystem is also a complex network in which multifarious species interact with each other to achieve system-level functions, such as nutrient biogeochemistry [1]. Microbial community is commonly considered as the primary driving force of ecosystem nutrient mobilization and metabolism, especially carbon (C), nitrogen (N), phosphorus (P), sulfur (S) and methane coupling process [2]. The rise of metagenomics and high-throughput array (e.g. PhyloChip, GeoChip, etc.) technologies enable acquiring the detailed information on microbial community functional gene abundance and diversity [3,4]; however, there has been far less attention focusing on the direct and indirect interactions between nutrient cycling genes coexisting in environmental samples. Documenting these interactions between functional genes across diverse microbial communities may help to clarify the functional roles and even environmental niches in different contexts [5]. With the increasing accumulation of functional gene data from modern high-throughput technologies, we are facing the challenge of those interaction explorations, and to extend analyses beyond sole abundance and diversity comparisons.

Network analysis, which originated from mathematicians and social scientists, is a useful mathematics tool based on statistical and structural analyses of item sets (nodes) and the connections between them (edges). Nowadays, network analysis has been widely accepted by biological scientists to explore molecular interactions in different metabolic pathways [6]. Moreover, various network methods (e.g., Bayesian network, co-expression network method, *etc.*) have been utilized to infer intercellular interactions based on gene expression array results [7]. The functionally genetic interaction within particular ecological processes (e.g., carbon dioxide elevation [8] and carbon sequestration [9]) or a microbial resistance system (e.g., antibiotic resistance gene [10]) has already been studied based on network analysis. However, network analysis was rarely used to study the co-occurrence patterns of functional genes across different biomes. Soil and water sediment are different habitats occupied by distinctive microbial communities, probably presenting sufficient difference in genetic abundances to resolve co-occurrence pattern networks. A successful application of co-occurrence pattern network analysis may offer new insight into the functionally structural generality and, perhaps, the similar co-dependent relationship between metabolic processes.

Recently published high-throughput functional gene quantification method QMEC (quantitative microbial elemental cycling) showed a significant absolute abundance difference in C, N, P, S and methane cycling gene structures (71 functional genes targeting 64 metabolism processes) between soil and water sediment samples [11]. The results from those samples (i.e., the same soils but with different crop hosts and the different-depth sediment in the same river estuary) could be used, in this study, to ascertain whether exists the similar co-occurrence patter of metabolic process across microbiomes. To investigate co-occurrence patterns of functional genes, each pairwise Spearman's rank correlations were calculated to construct correlation matrixes of all 71 functional genes. The *P*-value was adjusted with false discovery rate (FDR) correction for false-positive result reduction [12]. A correlation between two items was considered statistically robust if the Spearman's correlation coefficient (ρ) was >0.8 and *P*-value was <0.01 [13]. The resulting correlation matrixes were visualized with Gephi 0.9.1 [13].

Results showed both soil (Fig. 1a, c) and sediment (Fig. 1b, d) functional genes were intricately linked with each other, suggesting that C, N, P and S metabolic processes were tightly coupled

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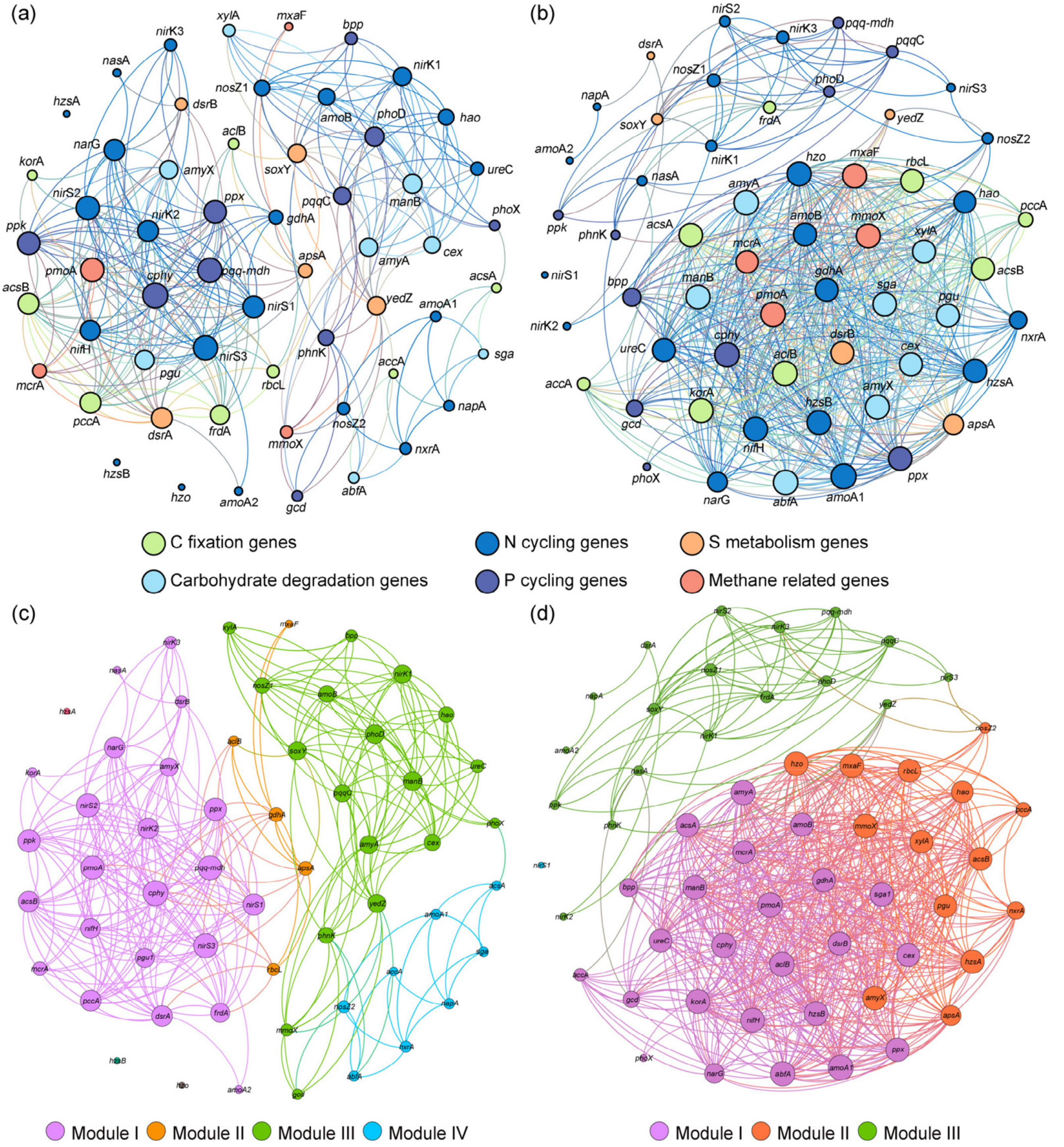

Fig. 1. The network analysis revealing the co-occurrence patterns between functional genes on both soil (a, c) and sediment (b, d). Based on different functionality (a, b) and modularity (c, d), the nodes were colored accordingly. A connection represents a strong (Spearman's correlation coefficient $P > 0.8$) and significant ($P > 0.01$) correlation. The size of each node is proportional to the amount of connections.

in both microbiomes. Based on functional classification, soil network (Fig. 1a) includes 54 nodes (anaerobic ammonia oxidation genes, *hzo*, *hzs*A and *hzs*B, were out of connection) and 301 edges with an average degree of 10.561, while the sediment network (Fig. 1b) consists of 56 nodes (nitrite reductase gene *nir*S1 was unconnected) and 604 edges with an average degree of 21.193. More connections (i.e., correlations between functional genes) observed in sediments than soils may indicate that sediment microbial community form a more tightened metabolic interaction network than soil for a better nutrient cycling. We speculate that a relatively lower nutrient status in sediment than soil may stimulate a better functional coordination of microbial community. A soil-sediment comparative research, which reported a significantly higher C, N, P and S eco-enzymatic activities in sediment than soil samples [14], could be used as a well supportive evidence that sediment habitat has a stronger metabolic interaction.

Both soil and sediment showed a small co-occurrence pattern (the average path distance for soil and sediment were 1.636 and 1.280 edges and the diameter for both were 5- and 4-edges, respectively), which means an efficient and rapid functional communication may happen among microbial members inside an ecosystem for rapid responses to outer-world disturbance (i.e., environmental changes). In another word, both soil and sediment microbial communities are robust ecosystems with functional stability. However, a smaller network also means a more vulnerable system. Microbial community functional unity of sediment, in this study, may be much easier affected by environmental perturbations than soil.

Modularity is an inherent property of network topological structure, which shows how the network was organized. From ecological view, modularity may reflect habitat heterogeneity, phylogenetic clustering of related species, and even functional association [10]. Obviously, the soil (Fig. 1c) and sediment (Fig. 1d) have different organizing pattern – they respectively have 4 and 3 major modules consisting of different functional genes. Additionally, based on modularity, we could easily clarify the gene hub (most connected node) in soil and sediment samples. Interestingly, phytase gene *cphy*, sulfur reductase gene *aps*A and sulfur oxidizing gene *sox*Y were common hubs of Module I, II and III in both soil and sediment environment, respectively. The identified hub could be further used as an effective indicator to qualitatively analyze the co-occurring functional genes. The presence of same functional gene in the same module may signify that the corresponding functional microorganisms have similar ecological niches. In this study, that also means phytase-hydrolyzer and S-cycler may serve as a unique and predominant functional representative or identifier across soil and sediment habitats.

In conclusion, using co-occurrence pattern network analysis, both soil and sediment have similar stable functional interactions in our study. Sediment microbial community has a much denser functional coordination but may easier receive environmental perturbations than soil. Although soil and sediment have different organizing patterns, both networks have the same functional identifiers, which may be considered as a common feature of soil and sediment functional structure.

Conflict of interest

The authors declare that they have no conflict of interest.

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