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Multi-faceted approaches to discovering and predicting microbial nutritional interactions

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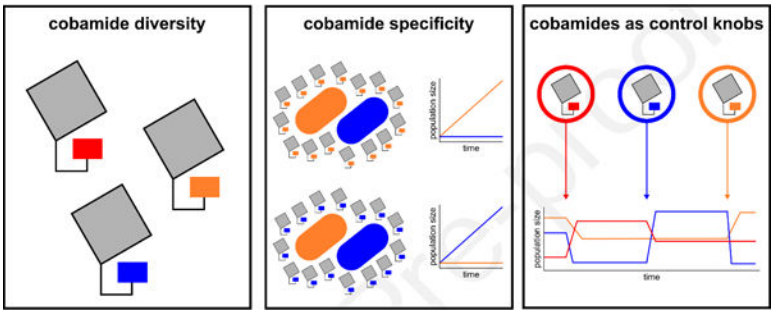
Abstract

Nearly all microbes rely on other species in their environment to provide nutrients they are unable to produce. Nutritional interactions include not only the exchange of carbon and nitrogen compounds, but also amino acids and cofactors. Interactions involving cross-feeding of cobamides, the vitamin B_{12} family of cofactors, have been developed as a model for nutritional interactions across species and environments. In addition to experimental studies, new developments in culture-independent methodologies such as genomics and modeling now enable the prediction of nutritional interactions in a broad range of organisms including those that cannot be cultured in the laboratory. New insights into the mechanisms and evolution of microbial nutritional interactions are beginning to emerge by combining experimental, genomic, and modeling approaches.

Graphical abstract



Introduction

In human society, virtually every individual relies on others for shelter, food, and other basic needs. Likewise, most microbes rely on others for shelter, in the form of a host organism or

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

biofilm structure, and food, such as carbon or micronutrients. Even many of earth's most self-sufficient microbes – photosynthetic aquatic microbes that are responsible for a substantial fraction of the planet's carbon fixation [1] – rely on neighboring bacteria for cross-protection from oxidative damage [2]. The least self-sufficient microbes depend on others for a multitude of physical and metabolic needs, which may explain why the majority of microbes have yet to be cultured in isolation [3,4].

Numerous studies of nutritional dependence have uncovered obligate mutualistic interactions between co-associated partners [5–11]. An extreme example is the interaction between insects and their bacterial endosymbionts. Here, bacteria and their hosts have evolved obligate metabolic interdependencies for amino acid and cofactor production, reflected in the presence of complementary metabolic pathways in their genomes [6,8,10]. Even a single metabolic pathway can be divided between multiple organisms that share biosynthetic precursors [10,12].

There is also abundant evidence for metabolic dependency among free-living microbes across all domains of life [3,12–18]. Carbon flow between microbes in different trophic levels is a major form of nutritional interaction in microbial ecosystems. Layered upon the flow of carbon is a complex suite of nutritional interactions involving the exchange of a variety of other metabolites [19]. Many studies focus on the sharing of carbon, nitrogen, or amino acids [8–10,13,15,17,18,20–22], but other types of metabolites such as cofactors are also frequently shared. Here, we review recent advances in experimental and computational approaches to investigate nutritional interactions, using the vitamin B_{12} family of corrinoid cofactors known as cobamides as an example. We then discuss a comprehensive modeling approach that can be used as a framework to study nutritional interactions across scales, from individual cells to the community.

Cobamides as model shared nutrients

Cobamide cofactors facilitate diverse biochemical reactions involved in methionine synthesis, nucleotide metabolism, natural product biosynthesis, and various pathways for carbon and nitrogen catabolism [23]. Cobamides are shared metabolites, as evidenced by the absence of cobamide biosynthesis genes in the majority of genomes of cobamide-utilizing organisms [24–26]. The widespread reliance on cobamide cross-feeding, coupled with their structural diversity and specificity (Fig. 1), makes cobamide sharing an ideal model to study nutritional interactions.

Cobamides consist of a central corrin ring, an upper ligand that directly participates in chemical reactions, and a structurally variable lower ligand (Fig. 1). Lower ligand structure is important for function, as enzymes of different species require different cobamides. The influence of cobamide structure on growth has been observed in microbes that depend on cobamides produced by other organisms, including bacteria from contaminated groundwater [27], human gut bacteria [28], and algae [29], suggesting that these microbes require strategies to acquire the specific cobamides that meet their nutritional needs. Indeed, some microbes have been shown to "remodel" cobamides, replacing the lower ligand with one that functions in their metabolism [27,29,30]. The ability of cobamide structure to alter growth

has also been documented in cobamide-producing bacteria that can be induced to produce non-native cobamides by incorporating exogenously supplied alternate lower ligands, a process known as guided biosynthesis [31–34].

Considering that half of bacteria are predicted to rely on cobamides produced by others, the abovementioned examples of cobamide specificity suggest that cobamide requirements can influence microbial partnerships and community population dynamics. The importance of cobamide specificity was demonstrated in a three-species consortium consisting of a cobamide-requiring predatory amoeba, a co-isolated cobalamin (vitamin B_{12})-producing *Pseudomonas* species, and a pseudocobalamin-producing cyanobacterium [35]. Grazing on the cyanobacterium alone could meet all of the amoeba's nutrient requirements except its cobamide requirement, which could only be satisfied by the *Pseudomonas* species due to its specific need for cobalamin. Similarly, in a three-species consortium containing the organohalide-respiring bacterium *Dehalococcoides mccartyi*, the cobamide-producing *Pelosinus* species synthesized a cobamide that could not be used by *D. mccartyi* [36]. As a result, the consortium could be cultivated only when an appropriate lower ligand base was added to allow cobamide remodeling by *D. mccartyi* [36]. The importance of cobamide specificity is also illustrated by the use of two distinct cobamides by cyanobacteria and eukaryotic algae in aquatic systems [29]. While pseudocobalamin produced by the cyanobacteria is available to cobamide-utilizing organisms, algae can only use it if they are able to remodel it to cobalamin. These findings highlight how cobamide diversity and specificity can create specific exchange networks between microbes. They also hint at a possible explanation for the structural diversity of a cofactor family with essentially the same set of enzymatic functions. Similar to the selective pressure on siderophore producers to diversify siderophore structure to limit piracy by other organisms [37], it is possible that structural diversity in cobamides emerged as a way to restrict the benefits of cobamide provisioning to specific partners [28].

The evolution and maintenance of cobamide dependence have been most extensively characterized in algal systems. Algal cobalamin auxotrophy is correlated with the presence of the cobalamin-dependent methionine synthase and absence of its cobalamin-independent counterpart. Loss of the cobalamin-independent methionine synthase has apparently arisen multiple times throughout evolution, likely due to co-evolution in stable association with a cobalamin-producing bacterial symbiont [14,38]. In support of this evolutionary mechanism, when the model alga *Chlamydomonas reinhardtii* was evolved in the laboratory in the presence of cobalamin, loss-of-function mutations in its cobalamin-independent methionine synthase gene arose, resulting in a growth advantage, but the mutations quickly reverted once cobalamin was removed [39]. These findings illustrate how the evolutionary tug-of-war between the cost of metabolite production and reliability of supply can be resolved by a stable metabolite-sharing partnership. Cobamide-requiring algae and cobamide-producing bacteria were indeed found to coexist stably under laboratory conditions [40]. Such partnerships can be stabilized by regulated reciprocal interactions. For example, bacterial growth in nutrient-poor aquatic systems is thought to be supported by shed algal cell wall components, while bacteria were found to supply cobalamin only when limited for carbon [14,40]. Another example of metabolic reciprocation was observed for the alga *Ostreococcus tauri* and the bacterium *Dinoroseobacter shibae* [12]. Here, in addition to carbon and

cobamides, a suite of B vitamins and B-vitamin precursors was exchanged. The stability of this partnership delicately relied upon reciprocation, as stability was lost once the B-vitamin and carbon requirements of the bacteria were decoupled from algal growth. Even though the mechanisms stabilizing such partnerships are beginning to be understood, it is still largely unclear how cobamide sharing in these natural systems evolved in the first place. For example, even basic questions including how cobamides are released from the cell, how export is regulated, and the costs associated with cobamide provisioning are still to be answered.

Predicting nutritional interactions by comparative genomics

In recent years, many examples of cobamide cross-feeding interactions have been discovered, as highlighted above. Nevertheless, such experimental investigations uncover only a tiny fraction of the total interactions occurring in nature. As an alternative to culture-based methods, genomic analyses can be used to gain insights into nutritional interactions on a broader scale. Because nearly all of the genes for cobamide biosynthesis and use have been characterized, the potential for cobamide-based interactions can be predicted by bioinformatics. An early study of 747 genomes (540 bacterial, 47 archaeal, and 160 eukaryotic) found that cobamide biosynthesis is present in 39% of bacteria, 72% of archaea, and is absent in all eukaryotes [26]. Similar results were reported in studies focusing on bacteria from the human gut and marine environments, with cobamide biosynthesis found in 40 and 48% of genomes, respectively [24,28,41]. Use of cobamide cofactors was found to be widespread in all of these studies, as indicated by the presence of cobamide-dependent enzymes in about two thirds of bacteria [26,28] and nearly all archaea [26].

The most recent genomic analysis of cobamide biosynthesis and utilization by Shelton et al. surveyed over 11,000 bacterial species, including 9% assembled from culture-independent metagenome and single-cell sequencing efforts [25]. Similar to previous studies, only 37% of bacteria were predicted to synthesize cobamides *de novo*, while 86% were predicted to use cobamides, confirming the prevalence of cobamide utilization and cross-feeding (Fig. 2). This study additionally showed that cobamide utilization varies among the four major phyla within the dataset, with Bacteroidetes and Proteobacteria having more cobamide dependent pathways than Actinobacteria and Firmicutes. More striking were disparities in the distribution of the cobamide biosynthesis pathway across phyla: it is present in 30–57% of genomes of Proteobacteria, Firmicutes, and Actinobacteria, but is absent in nearly all Bacteroidetes, despite the abundance of cobamide-dependent metabolism in this phylum (Fig. 2). An earlier analysis of 256 human gut bacterial genomes also found differences in cobamide biosynthesis capabilities across phyla, but unlike Shelton et al., predicted that approximately half of the 51 Bacteroidetes produce a cobamide, and that cobamide biosynthesis is nearly absent in Actinobacteria [24]. This disparity may reflect differences in the species composition and metabolism of the human gut compared to bacteria from all environments, could be due to incomplete sampling of the community, or may originate from subtle details used to predict biosynthetic capacities. While global observations such as the fraction of all analyzed bacteria predicted to produce cobamides were largely insensitive to methodological details of the studies [24–26,28,41], disparities at the phylum level highlight the inherent challenges in translating more detailed insights across different

systems and scales [25,26]. More generally, historic biases in the distribution of available genome sequences limit our ability to categorize biosynthetic capacities broadly across phylogenetic groups. For example, the four most abundant phyla investigated by Shelton et al. constituted 85% of the 11,000 species, with the majority of the remaining 108 phyla each being represented by fewer than 10 unique species [25].

Shelton et al. also identified a subset of genomes (17%) that contained partially complete cobamide biosynthesis pathways [25]. A small fraction of genomes (1.7%) contained a near-complete cobamide biosynthesis pathway, lacking only the initial 1–5 steps in the pathway. The authors experimentally verified this newly found class of precursor auxotrophy in three cases. Such experimental validation of genomics-based predictions is time-consuming but crucial for accurate estimation of metabolic capacities and nutritional interactions.

Exploring the evolution of cross-feeding via metabolic modeling

Culture-independent approaches, such as the genomic analyses discussed above and modeling approaches, can expand our knowledge of nutritional interactions without the need for time-intensive experimental analysis. Yet, the full breadth of nutritional interactions remains unknown, as modeling studies of nutrient exchange often do not explicitly account for the underlying interconnectedness of metabolic networks within cells [42,43]. To overcome these limitations, McNally and Borenstein employed a multi-layered modeling approach to examine the evolution of metabolite cross-feeding between two species, both represented by laboratory *Escherichia coli*, in the context of reductive evolution characterized by successive gene loss in both species [44]. This study applied flux balance analysis (FBA) to predict growth rates and metabolic fluxes within each species, in combination with a co-culture growth model that bridged the individual FBA models and an evolution model that incorporated gene loss (Fig. 3A). Cross-feeding interactions emerged in nearly 40% of over 16,000 simulated evolutionary trajectories, highlighting the vast potential for metabolite exchange in microbial metabolic networks. Revealingly, obligate cross-feeding interactions were frequently initiated by an initial phase of non-essential cross-feeding, suggesting that evolution of metabolic dependencies is derived from facultative sharing as an evolutionary steppingstone (Fig. 3B). Conversely, available shared nutrients were not always utilized, indicating “missed opportunities” for metabolic cooperation (Fig. 3B).

Deriving growth parameters and metabolite concentrations from an underlying mechanistic modeling layer [44,45] is an important step towards developing predictive models of growth and interaction dynamics in biological systems. In the future, models like the one used by McNally and Borenstein may lead to insights into how the interconnectedness of metabolism shapes behaviors at the cellular level by going beyond abstract representations of nutritional interactions. Ultimately, such predictions may allow investigators to plan, construct, and control biological systems in a framework similar to those used in mechanical or electrical engineering. Though the work by McNally and Borenstein is an important step towards such predictive frameworks, it also exposes the limitations of current metabolic models [44]. The inability to predict metabolic dependencies beyond three metabolites and the observed fitness decrease upon gene loss are, as pointed out by the authors, divergent from

experimental observations [10,12,46]. To overcome these limitations, future iterations of metabolic models must aim to encompass more biological reality and complexity, for example, by including gene regulation and expression dynamics, more stringently confining metabolic fluxes to experimental observations, and improving, and broadly evaluating, growth functions under various environmental conditions. Achieving such improvements will require a concerted experimental and modeling effort.

Conclusion

Recent advances in experimental, computational, and modeling approaches have uncovered a suite of novel insights into nutritional interactions across various scales, from individual metabolites, such as cobamides, to metabolic networks. Cobamides may be used in the future to evaluate or tune the composition and metabolism of microbial consortia. For example, cobamide-based chemical fingerprinting could be applied to track the metabolic function of a community, or cobamide supplementation could be employed to dynamically fine-tune relative species abundances in bioreactors. Applying metabolic modeling may facilitate the design of bioreactors that leverage detailed knowledge of cobamide biology to improve performance and robustness. We are now only beginning to understand the enormous potential of employing micronutrient exchange and metabolic specialization in biotechnological applications.

Acknowledgements

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References

1. Field CB, Behrenfeld MJ, Randerson JT, Falkowski P: Primary production of the biosphere: integrating terrestrial and oceanic components. science 1998, 281:237–240. [PubMed: 9657713]
2. Morris JJ, Johnson ZI, Szul MJ, Keller M, Zinser ER: Dependence of the cyanobacterium Prochlorococcus on hydrogen peroxide scavenging microbes for growth at the ocean's surface. PloS one 2011, 6:e16805. [PubMed: 21304826]
3. Razumov A: Direct Method for Bacteria Counting in a Sample. Its Comparison with Koch's Method. Mikrobiologiya 1932, 1:131–146.
4. Wrighton KC, Thomas BC, Sharon I, Miller CS, Castelle CJ, VerBerkmoes NC, Wilkins MJ, Hettich RL, Lipton MS, Williams KH: Fermentation, hydrogen, and sulfur metabolism in multiple uncultivated bacterial phyla. Science 2012, 337:1661–1665. [PubMed: 23019650]
5. Belzer C, Chia LW, Aalvink S, Chamlagain B, Piironen V, Knol J, de Vos WM: Microbial metabolic networks at the mucus layer lead to diet-independent butyrate and vitamin B12 production by intestinal symbionts. MBio 2017, 8:e00770–00717. [PubMed: 28928206] • The authors establish co-cultures of mucus-degrading and non-mucus degrading bacterial species and uncover cross-feeding of pseudo-cobalamin and propionate between Eubacterium hallii and Akkermansia muciniphila.
6. Duron O, Morel O, Noël V, Buysse M, Binetruy F, Lancelot R, Loire E, Ménard C, Bouchez O, Vavre F: Tick-bacteria mutualism depends on B vitamin synthesis pathways. Current Biology 2018, 28:1896–1902. e1895. [PubMed: 29861133] • The authors identify the importance of B-vitamin provisioning by a bacterial symbiont for tick development.
7. Graber JR, Breznak JA: Folate cross-feeding supports symbiotic homoacetogenic spirochetes. Appl. Environ. Microbiol 2005, 71:1883–1889. [PubMed: 15812016]

8. McCutcheon JP, Von Dohlen CD: An interdependent metabolic patchwork in the nested symbiosis of mealybugs. Current biology 2011, 21:1366–1372. [PubMed: 21835622]

9. Rakoff-Nahoum S, Foster KR, Comstock LE: The evolution of cooperation within the gut microbiota. Nature 2016, 533:255. [PubMed: 27111508]

10. Russell CW, Bouvaine S, Newell PD, Douglas AE: Shared metabolic pathways in a coevolved insect-bacterial symbiosis. Appl. Environ. Microbiol 2013, 79:6117–6123. [PubMed: 23892755]

11. Wexler AG, Schofield WB, Degnan PH, Folta-Stogniew E, Barry NA, Goodman AL: Human gut Bacteroides capture vitamin B12 via cell surface-exposed lipoproteins. eLife 2018, 7:e37138. [PubMed: 30226189] • The authors uncover a novel cobalamin-capturing mechanism in human gut associated Bacteroides. Surface exposed lipoproteins are found to be essential for cobalamin transport.

12. Cooper MB, Kazamia E, Helliwell KE, Kudahl UJ, Sayer A, Wheeler GL, Smith AG: Cross-exchange of B-vitamins underpins a mutualistic interaction between Ostreococcus tauri and Dinoroseobacter shibae. The ISME journal 2019, 13:334. [PubMed: 30228381] •• The authors demonstrate reciprocal B-vitamin cross-feeding between algae and bacteria. The algae provide niacin, biotin, and a precursor of folate, while the bacteria alleviate the thiamine and cobalamin auxotrophies of the algae.

13. Amin SA, Green DH, Hart MC, Küpper FC, Sunda WG, Carrano CJ: Photolysis of iron–siderophore chelates promotes bacterial–algal mutualism. Proceedings of the National Academy of Sciences 2009, 106:17071–17076.

14. Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG: Algae acquire vitamin B 12 through a symbiotic relationship with bacteria. Nature 2005, 438:90. [PubMed: 16267554] • The authors describe a mutualistic cross-feeding relationship between algae and bacteria. Algae obtain cobalamin from the bacteria in exchange for carbon. Bacterial cobalamin production is found to be upregulated in the presence of algae extract.

15. Foster RA, Kuypers MM, Vagner T, Paerl RW, Musat N, Zehr JP: Nitrogen fixation and transfer in open ocean diatom–cyanobacterial symbioses. The ISME journal 2011, 5:1484. [PubMed: 21451586]

16. Jiang X, Zerfaß C, Feng S, Eichmann R, Asally M, Schäfer P, Soyer OS: Impact of spatial organization on a novel auxotrophic interaction among soil microbes. The ISME journal 2018:1.

17. Kaiser C, Kilburn MR, Clode PL, Fuchslueger L, Koranda M, Cliff JB, Solaiman ZM, Murphy DV: Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. New Phytologist 2015, 205:1537–1551. [PubMed: 25382456]

18. Van Der Heijden MG, De Bruin S, Luckerhoff L, Van Logtestijn RS, Schlaeppi K: A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. The ISME journal 2016, 10:389. [PubMed: 26172208]

19. Zengler K, Zaramela LS: The social network of microorganisms—how auxotrophies shape complex communities. Nature Reviews Microbiology 2018:1.

20. Harcombe WR, Chacón JM, Adamowicz EM, Chubiz LM, Marx CJ: Evolution of bidirectional costly mutualism from byproduct consumption. Proceedings of the National Academy of Sciences 2018, 115:12000–12004.

21. Mee MT, Collins JJ, Church GM, Wang HH: Syntrophic exchange in synthetic microbial communities. Proceedings of the National Academy of Sciences 2014, 111:E2149–E2156.

22. Pande S, Merker H, Bohl K, Reichelt M, Schuster S, De Figueiredo LF, Kaleta C, Kost C: Fitness and stability of obligate cross-feeding interactions that emerge upon gene loss in bacteria. The ISME journal 2014, 8:953. [PubMed: 24285359]

23. Roth JR, Lawrence J, Bobik T: Cobalamin (coenzyme B12): synthesis and biological significance. Annual Reviews in Microbiology 1996, 50:137–181.

24. Magnúsdóttir S, Ravcheev D, de Crécy-Lagard V, Thiele I: Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. Frontiers in genetics 2015, 6:148. [PubMed: 25941533]

25. Shelton AN, Seth EC, Mok KC, Han AW, Jackson SN, Haft DR, Taga ME: Uneven distribution of cobamide biosynthesis and dependence in bacteria predicted by comparative genomics. The ISME journal 2019, 13:789. [PubMed: 30429574] •• The authors employ a comparative genomics

analysis to predict potential for cobamide biosynthesis and use in 11,000 bacterial species. Additionally, the study identifies and experimentally verifies a novel class of cobamide precursor auxotrophs.

26. Zhang Y, Rodionov DA, Gelfand MS, Gladyshev VN: Comparative genomic analyses of nickel, cobalt and vitamin B12 utilization. BMC genomics 2009, 10:78. [PubMed: 19208259]
27. Yi S, Seth EC, Men Y-J, Stabler SP, Allen RH, Alvarez-Cohen L, Taga ME: Versatility in corrinoid salvaging and remodeling pathways supports corrinoid-dependent metabolism in Dehalococcoides mccartyi. Appl. Environ. Microbiol 2012, 78:7745–7752. [PubMed: 22923412]
28. Degnan PH, Barry NA, Mok KC, Taga ME, Goodman AL: Human gut microbes use multiple transporters to distinguish vitamin B12 analogs and compete in the gut. Cell host & microbe 2014, 15:47–57. [PubMed: 24439897]
29. Helliwell KE, Lawrence AD, Holzer A, Kudahl UJ, Sasso S, Kräutler B, Scanlan DJ, Warren MJ, Smith AG: Cyanobacteria and eukaryotic algae use different chemical variants of vitamin B12. Current Biology 2016, 26:999–1008. [PubMed: 27040778] • The authors demonstrate that the two major phytoplankton groups utilize different forms of cobamides. Furthermore, the study demonstrates the potential of cobamide remodeling by algae, highlighting an additional complexity of nutrient fluxes within aquatic communities.
30. Gray MJ, Escalante-Semerena JC: The cobinamide amidohydrolase (cobyric acid-forming) CbiZ enzyme: a critical activity of the cobamide remodelling system of Rhodobacter sphaeroides. Molecular microbiology 2009, 74:1198–1210. [PubMed: 19889098]
31. Crofts TS, Seth EC, Hazra AB, Taga ME: Cobamide structure depends on both lower ligand availability and CobT substrate specificity. Chemistry & biology 2013, 20:1265–1274. [PubMed: 24055007]
32. Keller S, Ruetz M, Kunze C, Kräutler B, Diekert G, Schubert T: Exogenous 5, 6-dimethylbenzimidazole caused production of a non-functional tetrachloroethene reductive dehalogenase in S ulfurospirillum multivorans. Environmental microbiology 2014, 16:3361–3369. [PubMed: 24433392]
33. Mok KC, Taga ME: Growth inhibition of Sporomusa ovata by incorporation of benzimidazole bases into cobamides. Journal of bacteriology 2013, 195:1902–1911. [PubMed: 23417488]
34. Yan J, Şimşir B, Farmer AT, Bi M, Yang Y, Campagna SR, Löffler FE: The corrinoid cofactor of reductive dehalogenases affects dechlorination rates and extents in organohalide-respiring Dehalococcoides mccartyi. The ISME journal 2016, 10:1092. [PubMed: 26555247]
35. Ma AT, Beld J, Brahamsha B: An amoebal grazer of cyanobacteria requires cobalamin produced by heterotrophic bacteria. Appl. Environ. Microbiol 2017, 83:e00035–00017. [PubMed: 28283521] • The authors describe a three-species community of an amoeba, a cyanobacterium, and a Pseudomonas strain. The amoeba requires not only cyanobacteria to graze on, but also cobalamin produced by Pseudomonas.
36. Men Y, Seth EC, Yi S, Allen RH, Taga ME, Alvarez-Cohen L: Sustainable growth of Dehalococcoides mccartyi 195 by corrinoid salvaging and remodeling in defined lactate-fermenting consortia. Appl. Environ. Microbiol 2014, 80:2133–2141. [PubMed: 24463969]
37. Barber MF, Elde NC: Buried Treasure: Evolutionary Perspectives on Microbial Iron Piracy. Trends Genet 2015, 31:627–636. [PubMed: 26431675]
38. Helliwell KE, Wheeler GL, Leptos KC, Goldstein RE, Smith AG: Insights into the evolution of vitamin B12 auxotrophy from sequenced algal genomes. Molecular biology and evolution 2011, 28:2921–2933. [PubMed: 21551270]
39. Helliwell KE, Collins S, Kazamia E, Purton S, Wheeler GL, Smith AG: Fundamental shift in vitamin B 12 eco-physiology of a model alga demonstrated by experimental evolution. The ISME journal 2015, 9:1446. [PubMed: 25526368] • The authors perform experimental evolution of a model alga in the presence of cobalamin. A transposon insertion inactivates the cobalamin-independent methionine synthase METE in one replicate, resulting in increased fitness. Upon removing cobalamin the mutation quickly reverts.
40. Kazamia E, Czesnick H, Nguyen TTV, Croft MT, Sherwood E, Sasso S, Hodson SJ, Warren MJ, Smith AG: Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation. Environmental microbiology 2012, 14:1466–1476. [PubMed: 22463064]

41. Heal KR, Qin W, Ribalet F, Bertagnolli AD, Coyote-Maestas W, Hmelo LR, Moffett JW, Devol AH, Armbrust EV, Stahl DA: Two distinct pools of B12 analogs reveal community interdependencies in the ocean. Proceedings of the National Academy of Sciences 2017, 114:364–369.• The authors identify two distinct cobamide pools in the surface ocean, cobalamin and pseudo-cobalamin. The two cobamides have distinct bacterial source and selectively support enzyme activity.

42. Estrela S, Morris JJ, Kerr B: Private benefits and metabolic conflicts shape the emergence of microbial interdependencies. Environmental microbiology 2016, 18:1415–1427. [PubMed: 26287440]

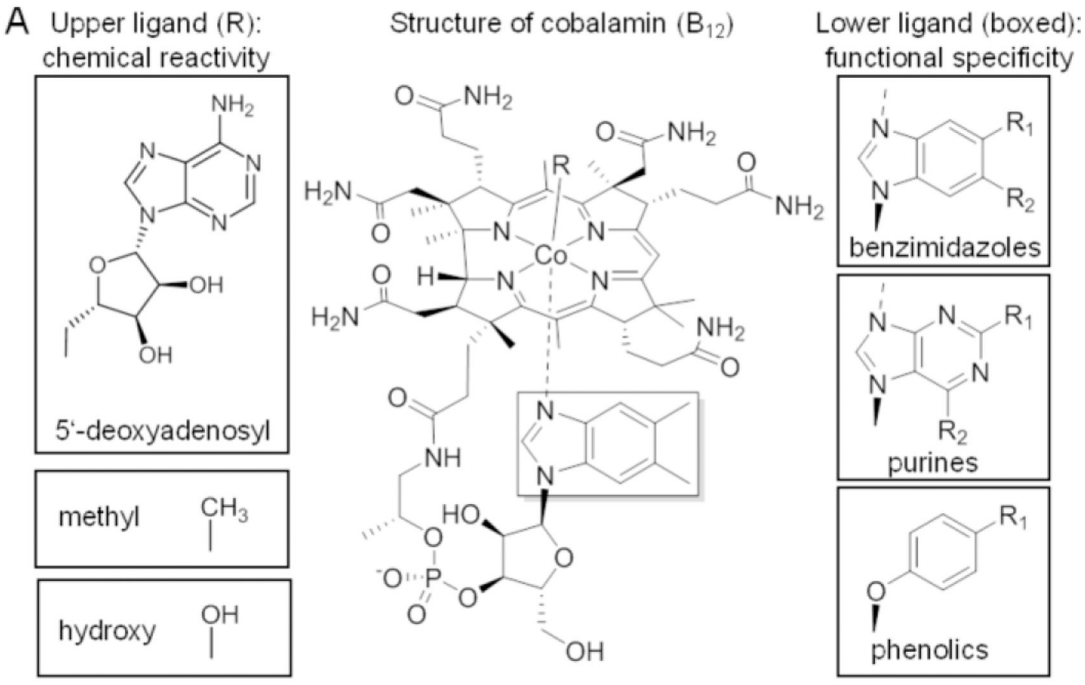
43. Oliveira NM, Niehus R, Foster KR: Evolutionary limits to cooperation in microbial communities. Proceedings of the National Academy of Sciences 2014, 111:17941–17946.

44. McNally CP, Borenstein E: Metabolic model-based analysis of the emergence of bacterial cross-feeding via extensive gene loss. BMC systems biology 2018, 12:69. [PubMed: 29907104] •• The authors employ metabolic modeling to simulate evolution of nutrient sharing in two-species communities undergoing massive gene loss. Evolution of cross-feeding is found to be widespread and signatures of pathway complementation are observed.

45. Chiu H-C, Levy R, Borenstein E: Emergent biosynthetic capacity in simple microbial communities. PLOS computational biology 2014, 10:e1003695. [PubMed: 24992662]

46. D'Souza G, Waschina S, Pande S, Bohl K, Kaleta C, Kost C: Less is more: selective advantages can explain the prevalent loss of biosynthetic genes in bacteria. Evolution 2014, 68:2559–2570. [PubMed: 24910088] •• The authors experimentally quantify fitness effects for loss of biosynthetic genes for amino acids, nucleobases, and vitamins. Generally, auxotrophs outcompete prototrophs when the required metabolite is supplemented. Fitness effects are found to be concentration-, gene-, and strain-specific.

47. Tanioka Y, Miyamoto E, Yabuta Y, Ohnishi K, Fujita T, Yamaji R, Misono H, Shigeoka S, Nakano Y, Inui H, et al.: Methyladeninylcobamide functions as the cofactor of methionine synthase in a Cyanobacterium, Spirulina platensis NIES-39. FEBS Lett 2010, 584:3223–3226. [PubMed: 20558164]



B

Bacteroides thetaiotaomicron	✓	✓	✗
Dehalococcoides mccartyi	✓	✗	✗
Sinorhizobium meliloti	✓	✗	✗
Sporomusa ovata	✗		✓
Arthrospira platensis	✗	✓	
Ostreococcus tauri	✓	✗	

Figure 1. Structural and functional diversity of cobamides.

A. Structure of cobalamin (vitamin B_{12}) and various upper and lower ligands of cobamides. Cobamides consist of a central corrin ring, an upper ligand (R), and a lower ligand (boxed), covalently attached via the nucleotide loop. Upper ligands confer chemical activity by generating a radical or donating a methyl group. Lower ligands are diverse and often do not directly participate in chemical reactions, but confer enzyme specificity. **B.** Examples of specificity of microbes for three cobamides with the lower ligands shown. Check mark indicates cobamides that can be used; X indicates cobamides that poorly support growth or enzyme activity; blanks indicate cobamides that were not tested [27–29,31,33,47].

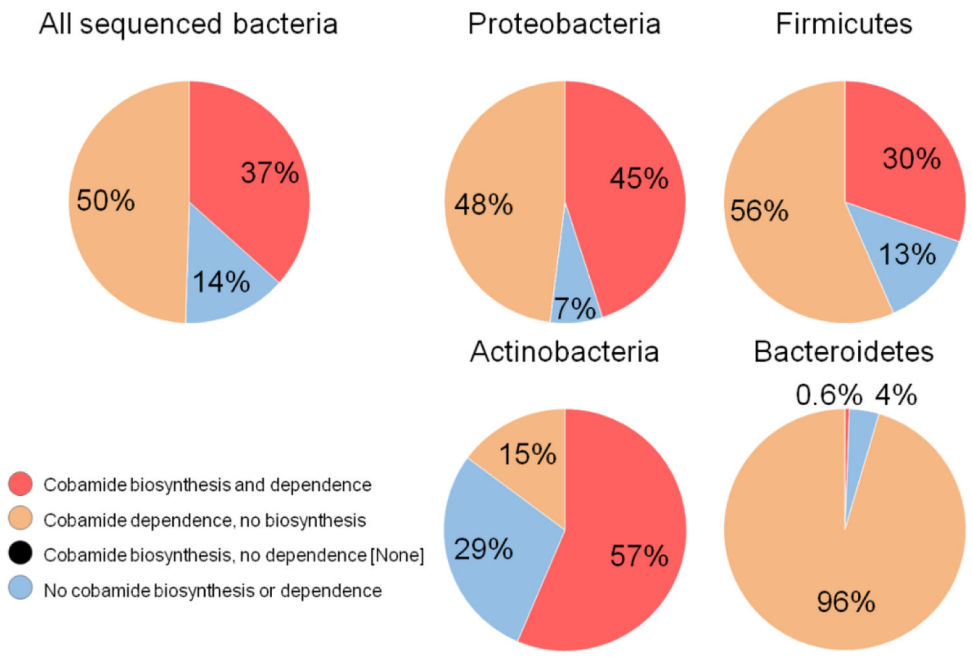


Figure 2. Uneven distribution of cobamide production and use in bacteria.

Distribution of the *de novo* cobamide biosynthesis pathways and cobamide-dependent pathways among all bacteria (left) and for the four most abundant phyla in the dataset. Percentages larger than 1 are rounded to the closest integer. Adapted from [25].

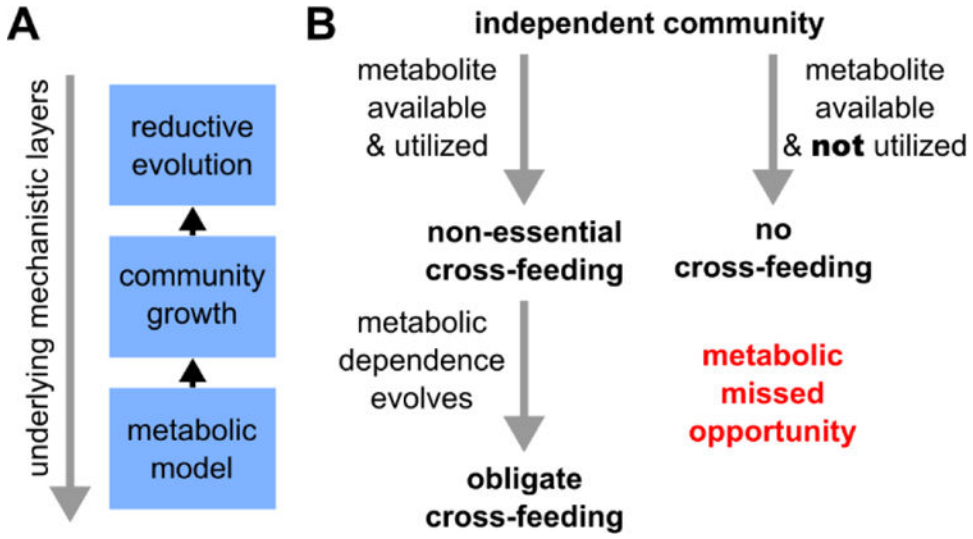


Figure 3. Multi-layered framework for modeling the evolution of cross-feeding in a co-culture undergoing successive gene loss.

A. Three-layered framework to model evolution of cross-feeding interactions in microbial metabolic networks. Flux balance analysis (FBA) is employed to iteratively predict instantaneous growth, metabolite uptake and metabolite secretion rates of each species (bottom). The behavior of the individual species is fed into a co-culture model to simulate community growth in a shared environment allowing for metabolite exchange (middle). Reductive evolution is performed by iteratively deleting metabolic genes at random (excluding genes with major fitness defects) until no more genes that meet this condition can be removed from either species (top). **B.** Schematic illustration of the development of cross-feeding (left) and metabolic missed opportunities (right). Panel A adapted from [44].