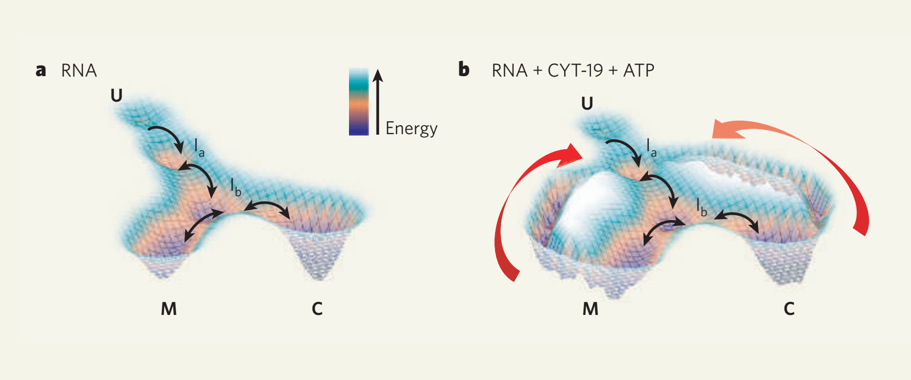

Figure 1 | Schematic folding landscape of group-I intron RNAs. In this representation, valleys indicate structures of varying stability — the deeper a valley, the lower the energy of the structure and the more stable it is. The ridges between valleys correspond to rates of inter-conversion between neighbouring structures; high ridges indicate slow rates. U represents an unfolded RNA, I_a and I_b are intermediate structures, M is a misfolded and C a correctly folded RNA. **a**, Left to their own devices, most group-I intron RNAs first form M, which is less stable than C but forms more quickly. However, M converts back into I_b and I_a at an appreciable rate, allowing further rounds of folding into both C and M. Although C can also convert back to I_b, this process is slow. So with time, C accumulates. **b**, Bhaskaran and Russell[3] show that CYT-19 alters the RNA-folding landscape in an ATP-dependent manner. By unfolding both M and C, new pathways for structural conversions are opened that presumably lead to I_a/I_b (red arrows). The formation of the RNA species depends on the rates at which they can interconvert. This represents a kinetically controlled steady state, in which less stable structures can accumulate. The levels of ATP determine and maintain the topology of the folding landscape, and in the absence of ATP the landscape changes back to that seen in **a**.

structure are readily unfolded by CYT-19. So it is possible that the reason for the slower unfolding rate of correctly folded RNAs is that crucial helices are sequestered in the higher-order structure and are thus protected from unwinding by CYT-19. This possibility is supported by an earlier study[7] showing that CYT-19 cannot easily unwind duplexes hidden within an RNA structure. If this is true for other nonspecific DEAD-box chaperones, this would be an elegant and unexpectedly simple way by which these proteins might preferentially unravel misfolded RNA structures.

Bhaskaran and Russell also make another intriguing observation. Under certain conditions — for example, at reduced magnesium concentrations, a consequence of which is the weakening of higher-order RNA structures — CYT-19 increases the ratio of misfolded RNAs to correctly folded structures. This is remarkable, because misfolded group-I RNAs are thermodynamically less stable than the correct structures and, at equilibrium, the most stable species dominates in a mixture of possible structures.

The authors find that CYT-19 achieves the redistribution of RNA species against their thermodynamic equilibrium by establishing a 'kinetically controlled steady state' (Fig. 1b). In this state, CYT-19 constantly unwinds RNA structures in an ATP-dependent manner, so that the distribution of these structures is no longer dictated by their thermodynamic stabilities but, instead, by the rate at which they are actively unravelled by CYT-19, as well as by the rate of their refolding. Under these conditions, CYT-19 readily unravels both misfolded and correct structures. But when the RNA subsequently misfolds it does so faster than correctly refolding RNA, and therefore accumulates.

Similar ATP-driven redistribution of model RNA complexes by another DEAD-box protein has been reported[8]. So it seems that DEAD-box proteins can establish kinetically controlled steady states for various RNAs. The ability of these proteins to favour the accumulation of thermodynamically less stable RNA species seems particularly beneficial for processes in which RNAs must undergo extensive structural changes, such as during ribosome assembly and pre-messenger-RNA splicing. Both of these processes involve numerous DEAD-box proteins[4].

The work of Bhaskaran and Russell[3] implies that sequence nonspecificity of DEAD-box proteins is more of an asset than a disadvantage, at least for their function as RNA chaperones. Nonspecific chaperone proteins can work on many misfolded RNAs and at different positions within them, which obviates the need for a large number of highly specific proteins that would exclusively aid the folding of each of the many cellular RNAs. The potency of unfolding by the omnipresent and nonspecific DEAD-box proteins also offers one possible explanation for why some RNAs tolerate misfolding into fairly stable structures. This is because they can rely on DEAD-box proteins to rescue them. But an increased awareness of the versatility of nonspecific DEAD-box proteins highlights the next question: why does the cell use so many of these enzymes? ■

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ENVIRONMENTAL SCIENCE

Nutrients in synergy

Eric A. Davidson and Robert W. Howarth

A literature meta-analysis of the effects of nitrogen and phosphorus on plant growth prompts a thought-provoking inference — that the supply of, and demand for, these nutrients are usually in close balance.

The nutritional needs of plants start with carbon, hydrogen and oxygen, which they obtain from atmospheric gases and from water. They also require nitrogen (a constituent of all proteins) and phosphorus (not least as a component of nucleotides, including those in DNA and RNA). Writing in *Ecology Letters*, Elser and colleagues[1] provide a timely contribution to our understanding of how nitrogen and phosphorus, both individually and in combination, affect primary producers such as crops, trees and algae.

This is a topic with a long history. In the nineteenth century, Justus von Liebig proposed his 'law of the minimum', which describes crop production as being limited by the nutrient in shortest supply. According to this law, once the nitrogen (N) need of a crop is met by fertilization, another element such as phosphorus (P) becomes limiting (Fig. 1a), and there is no further response to additional N.

Since Liebig's day, the use of N and P fertilizers has revolutionized agriculture. The consequences have been both desirable and undesirable. Synthetic fertilizers have fuelled the Green Revolution and greatly lessened world hunger and malnutrition. But they have also led to severe air and water pollution and other adverse effects on the environment and human health[2,3]. The widespread distribution of excess N and P within natural ecosystems has caused degradation of coastal waters globally, and an increase in hypoxic areas ('dead zones') on nearly every continent[3,4]. Excess N and/or P lead to algal blooms and so consumption of the oxygen required for productive fisheries and for healthy marine habitats. In the United States, two-thirds of coastal rivers and bays are degraded through the consequences of excess nutrients[5]. Similar problems occur in fresh waters, and excess fertilization of forests can lead to decline and loss of biodiversity. Management strategies for mitigating the two pollutants can differ[2]. So the long-standing debate over the relative importance of N and P as agents of excess production in different ecosystems is of immense practical significance.

This is the context in which Elser and colleagues' study[1] is set. It is a meta-analysis of more than 300 publications reporting results of nutrient-amendment experiments in marine, freshwater and terrestrial ecosystems, with the effects of the two elements being assessed in terms of increased biomass or production. The study supports some well-established rules of thumb in biogeochemistry. Examples are the greater limitation on P than N in mature forests growing on highly weathered lowland soils in the tropics; the greater responses to P than N addition in freshwater ecosystems; and the greater responses to N addition in marine ecosystems.

More importantly, the analysis demonstrates a surprisingly consistent pattern of a synergistic effect of N and P addition on net primary productivity across all ecosystem types. Adding N and P together seems to give photosynthesis by algae and higher plants more of a boost than adding either one separately. The authors infer from this that the stoichiometry of N and P supply and demand must generally be in close balance in most ecosystems. According to this interpretation, P is rarely available in great excess relative to N, so a modest addition of N quickly provokes a limitation on P. When N and P are added together, N and P limitation may alternate in numerous small incremental steps, ultimately producing a synergistic effect (Fig. 1b).

This is an impressive synthesis[1], but several caveats are in order. First, we lack a mechanistic understanding of how the availability of one resource affects the supply of and demand for another resource. At the cellular level, regulation of the relative dynamics of the demand for N for the synthesis of enzymes, and the demand for P for the synthesis of nucleic acids and also of ATP, is poorly known. At the organismal level, some species have adaptations for obtaining a nutrient that would otherwise be difficult to acquire (for example, diverting carbon and nitrogen to the fungal mycorrhizae that form symbioses with plant roots to improve access to P in nutrient-poor soils). But our knowledge of such trade-offs is only qualitative. And at the ecosystem level, the factors that may influence the relative importance of N fixation from the atmosphere, such as the availability of N, P and molybdenum (an essential component of N-fixing enzymes), and competition for light and water, are not well understood[6].

Second, the doses of N and P addition — either through natural pulses or in bioassay experiments — may be very important, but were not specifically addressed by Elser and colleagues. If the doses are large enough, adding both nutrients may simply alleviate first one nutrient limitation and then the other, as per Liebig's classic law, and this could look like a synergistic effect. The assertion that the stoichiometric supply of N and P in natural systems is generally close to balanced could be further tested by quantitative dose–response experiments.

Third, there is the issue of the timescale of nutrient-amendment experiments. Experiments that are short in duration relative to the life cycles of the organisms being studied measure only the response of the organisms that are dominant in the ecosystem at the time of the assay[5,7]. Longer-term ecosystem-scale responses to nutrients can be different, as the dominant species change with changes in biogeochemical processes. A short-term assay in a freshwater lake, for example, would probably indicate P limitation, followed by an apparent synergistic effect with N limitation once enough P were added. But in one experiment[8], years of P fertilization led to a predominance of N-fixing cyanobacteria that produced enough reactive nitrogen to keep the lake P limited.

Finally, resource limitation may simultaneously involve several nutritional elements, along with light, water and carbon dioxide. If responses to the addition of N and P are synergistic, we would expect complex synergies among other potential limiting resources as well.

So we are left with plenty of questions. Nonetheless, Elser and colleagues' meta-analysis[1] provides the most thorough examination of short- and medium-term nutrient-amendment experiments to date. Their synthesis adds to a growing body of evidence that addressing the 'off-farm' environmental consequences of food production will require efforts to reduce losses of both N and P from agricultural systems[2,5,7]. It also provides an improved basis for formulating testable hypotheses to describe nutrient interactions in other ecosystems. ■

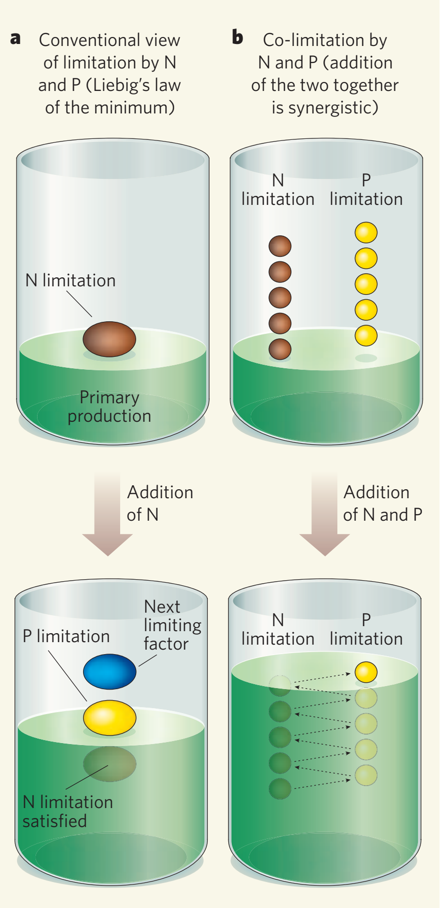

Figure 1 | Nutrients and primary production. a, The conventional view of Liebig's law of the minimum. Here, after the need for nitrogen (N) is met, N is not limiting, and increased production successively depends on satisfying limitation by phosphorus (P) and then any other limiting factors. **b,** A modification of the law of the minimum, based on one interpretation of the synthesis by Elser *et al.*[1]. From their meta-analysis, they infer that supply and demand of N and P are usually in close balance, so that an incremental addition of one causes a modest limitation by the other. Alleviation of each incremental limitation in turn produces a synergistic effect when both nutrients are added together.

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