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Addressing Vitamin B_{12} deficiency through aeroponic fortification of a salad crop (*Pisum sativum*)

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Abstract

Plants do not produce Vitamin B_{12}, creating a nutrient insufficiency risk for those who do not consume animal-derived foods. Furthermore, various diseases cause Vitamin B_{12} deficiency. Here, we establish an approach for B_{12} dietary supplementation that harnesses a horticultural technology to deliver the recommended daily allowance (RDA) of B_{12} within a single portion of a salad crop (pea shoots). We demonstrate the effectiveness of the approach in a commercial and scalable growing environment, conducted an economic evaluation, find that it has versatility for growers, does not alter the product shelf-life, and that the B_{12} persists during cold-chain storage. Furthermore, the RDA of B_{12} is bioaccessible from this crop during simulated human digestion. Taken together, this provides a commercially-viable approach for dietary supplementation of B_{12} intake, and a roadmap for the development and evaluation of fortification strategies.

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Introduction

Vitamin B_{12} is the only essential vitamin that is absent from plants, and therefore represents a nutritional insufficiency risk as people adopt more sustainable diets that exclude meat[1]. Humans use the nutrient as a cofactor for just two enzymes, methionine synthase and methylmalonyl-CoA mutase[2]. The very long half-life of B_{12} means that the nutrient is required in only minute quantities daily[2-5], with the USA recommended daily allowance (RDA) for B_{12} being 2.4 µg/day for adults aged 16 – 65. Despite this low requirement for the vitamin, an estimated 6% of the UK adult population is B_{12} deficient (i.e. ≤ 150 pM in serum) and a further 44% of the UK population is in the marginal or insufficient range (150-258 pM)[6].

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Clinical hallmarks of B_{12} deficiency are anaemia and neurological dysfunction of varying severity[2-4,7,8], as the nutrient is implicated in metabolic roles associated with the formation of red blood cells, myelin sheet formation, and neutotransmitter synthesis. Apart from dietary insufficiency, B_{12} deficiency can also result from autoimmune gastritis, often referred to as pernicious anaemia, genetic factors associated with absorption, transport and activation, infections and parasites, and recreational drug use.

B_{12} is the most structurally complex nutrient that is made exclusively by specific prokaryotes, and is completely absent from multicellular plants. While ruminants including cows absorb B_{12} produced by prokaryotes within their digestive tracts[9], the human gut microbiome appears to supply little, if any, B_{12} to its host. Therefore, humans mainly acquire their B_{12} from animal-based foods in their diet[3,4,10]. The major dietary sources of B_{12} include fish, meat, poultry, eggs, milk, and other dairy products. Shellfish and algae are also thought to contain sources of B_{12}, but it is unclear whether these products contain analogues of B_{12} that are active to humans[11,12]. B_{12} forms that are processed and/or used directly by human cells include methylcobalamin, adenosylcobalamin, hydroxycobalamin and cyanocobalamin[13]. The biochemical synthesis of B_{12} requires around thirty enzyme-mediated steps, which negates any possibility of engineering the biosynthetic pathway into edible plants in the near future.

Despite the presence of B_{12} in many dietary sources, it is important for those that are B_{12} deficient or insufficient to supplement their nutrition with B_{12}. However, tablet-based B_{12} supplements are often ingested without food, which might prevent optimal B_{12} absorption because intrinsic factor (a glycoprotein that complexes with B_{12} and is essential for its uptake) is secreted by gastric parietal cells in response to eating[14,15]. An alternative approach to B_{12} tablets is fortified food products, which can be consumed as part of a nutritious diet that promotes optimal B_{12} absorption. This includes fortification of a variety of foods, such as breakfast cereals,

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milk, and wheat flour[2], with B_{12} fortification of flour occurring in many countries[16]. An alternative approach considered here is the fortification of fresh salad products.

The consumption of young salad leaves has gained popularity[17,18], with these salads reported to have enhanced nutritional profiles and contributing to dietary diversification[19]. For example, the microgreens market was valued at $1,276.0 million in 2019 and is estimated to reach $2,049.3 million by 2028, registering a compound annual growth rate of 11.1% from 2021 to 2028 (not inflation-adjusted)[20]. Within this market, there is a desire from growers and supermarkets to produce more nutritious salad crops, to add value and health-benefit differentiators to their products whilst fulfilling an important social role. We reasoned that there is potential to fortify salad products with B_{12} during cultivation, since B_{12} can be absorbed by certain plants when present in the growth medium[21-26] (Supplementary Table 1). Furthermore, existing patents indicate that some B_{12} can be absorbed by the seeds of various crops[27,28]. However, it is unclear whether approaches that involve supplementing plant growth media with B_{12} leads to a salad product that contains the RDA of B_{12}, whether it can be absorbed by human digestion, and whether such approaches are economically viable or scalable. Here, we developed an approach to fortify pea shoots with B_{12} within a commercial growing environment, exploiting the rapid root development that occurs in aeroponic horticulture[29] to deliver bioaccessible RDA of B_{12} within a single salad portion.

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Results

Aeroponic fortification of pea shoots with Vitamin B_{12}

We evaluated whether salads might be supplemented with B_{12} during aeroponic horticulture in indoor farms, using pea shoots as an experimental model. We selected pea shoots (*Pisum sativum* CN Seeds cultivar 4019*)* because these represent a desirable crop for growers, as they have a rapid growth cycle, being ready for harvest within 10 days of germination in indoor

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horticultural environments, and this variety has tendril-free morphology that is better suited to industrial packing. Single or mixed pea shoot-containing salad bags are relatively popular with consumers because they add flavour compared to salads such as lettuce. A typical supermarket bag of pea shoots is 60-80 g, and we considered 15 g of pea shoots to be a single meal portion per adult when comparing fortification levels to the RDA.

We conducted this work in a vertical farm, using a commercially available aeroponics growing platform (Fig. 1a, b). Although such environments have greater environmental variability than laboratory-based experimental plant growth chambers, we selected this approach to evaluate the scalability of our approach. During cultivation, the aeroponically-supplied nutrient solution was supplemented with a form of B_{12}, cyanocobalamin, at various growth stages (Fig. 1a, b). We chose to use cyanocobalamin rather than other forms of the nutrient, because this is the most widely available form that is also bioactive for humans and has greater stability than adenosylcobalamin and methylcobalamin, which photodegrade in seconds[30]. Because B_{12} can be absorbed by seeds that are soaked in B_{12} prior to germination[27] and can accumulate within the cellular vacuoles of some plant species[21], we reasoned that aeroponically cultivated pea shoots might absorb B_{12} through their roots and accumulate B_{12} within harvestable plant shoots. Furthermore, the aeroponic cultivation method leads to substantial root growth (Fig. 1b) and can increase root hair formation[31], potentially maximising interaction between the fortified solution with plant roots.

We supplemented the aeroponic cultivation nutrient solution with a range of cyanocobalamin concentrations, cultivated the plants for 8 days, and then harvested material for B_{12} quantification using LC-MS analysis. We chose LC-MS rather than other methods because this can distinguish the between the cobalamin forms that have human nutritional benefit and those that do not. In parallel, we replicated an existing seed-soaking method[27] and measured the quantity of B_{12} within harvestable tissue. Supplementation of the aeroponic bed with

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cyanocobalamin for 48 h after transfer of seedlings to the growing system led to the accumulation of cyanocobalamin within the aerial portion of the plants (Fig. 2b). The quantity of B_{12} that eventually accumulated reflected the dosing concentration (Fig. 2b). Supplementation with 10 μM of cyanocobalamin during aeroponic cultivation led to the accumulation of approximately 0.17 μg B_{12} per gram of pea shoot tissue (Fig. 2b). In comparison, in a parallel experiment where seeds were soaked in cyanocobalamin solution prior to germination and cultivation[27], around 0.02 μg B_{12} accumulated per gram of pea shoot tissue when cyanocobalamin was supplied at 10 μM (Fig. 2b). Therefore, a considerably greater quantity of B_{12} accumulated within aerial plant tissue after B_{12} supplementation during aeroponic cultivation, compared with an existing seed-soaking patent[27]. Using these data, we calculated the quantity of pea shoots that need to be harvested to obtain plant tissue that contains the US adult RDA of B_{12} (2.4 μg/day), based on the B_{12} levels accumulated during cultivation. At the greatest cyanocobalamin concentration tested (10 μM), approximately 18 g of pea shoots contained the US B_{12} RDA (Fig. 2b), raising the possibility that consumption of a relatively small quantity of pea shoots cultivated using this approach might deliver the RDA of B_{12}. In contrast, it would be necessary to consume > 1.2 kg of pea shoots to obtain the RDA of B_{12} from plants generated using the seed-soaking method (using 10 μM B_{12}; Fig. 2b), which seems unrealistic.

To optimize our approach, we were interested to identify the pea shoot tissues in which the B_{12} accumulated during aeroponic supplementation with cyanocobalamin. Therefore, we conducted an experiment in which pea shoots supplemented with a variety of cyanocobalamin concentrations were separated into the germinated seeds, stems, leaves and roots, and the B_{12} content was measured in pooled tissue from each of these organs. The greatest B_{12} quantities accumulated within the leaves and roots (Fig. 2c, d), little was detected in the stems, and, in one independent repeat (Fig. 2c), some B_{12} was present in the remains of germinated seeds. Although the overall B_{12} accumulation varied between experimental repeats (Fig 1C, D), a

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consistent feature was that B_{12} supplementation at 10 μM and 20 μM cyanocobalamin led to sufficient B_{12} accumulation within the leaves to meet the RDA of B_{12} in a portion of 15 g of leaf tissue. The roots also accumulated B_{12} (Fig 1C, D), but pea root tissue is unlikely to be attractive or palatable for consumers. This means that there would be some wastage of the B_{12} molecule; if this represented an economic concern when growing at scale, future methods could be developed to liberate and reuse this B_{12} from root tissue.

To examine variation in B_{12} accumulation within leaf tissue, we conducted 5 independent growing trials, supplementing the plants with 10 μM cyanocobalamin, and compared B_{12} accumulation in the leaves after 8 days of growth (Fig. 2e). The mean B_{12} content always exceeded the USA RDA (2.4 μg) in 15 g of leaf tissue, although there was significant variation in B_{12} levels between trials at the time of harvest (Fig. 2e). This variation could be due to factors such as gradients of environmental conditions, edge effects, and planting density variation in the commercial growing environment. This is an important finding as it demonstrates that a cyanocobalamin dosing concentration at or exceeding 10 μM is sufficient to reach the B_{12} RDA threshold irrespective of environmental variation across a commercial growing environment. Taken together, these experiments demonstrate that the leafy tissue of pea shoots supplemented with cyanocobalamin consistently accumulates the RDA of B_{12} within a single 15 g salad portion.

Flexibility of Vitamin B_{12} fortification across pea shoot developmental stages

We determined that it is possible to achieve the RDA of B_{12} within a relatively small quantity of pea shoots cultivated using aeroponics. We reasoned that it might be beneficial for growers to have flexibility in the developmental stage at which the plants are supplemented with B_{12} during their cultivation. To investigate this, we cultivated pea shoots using the aeroponic platform and supplemented their growing media with cyanocobalamin for 48 h at three different developmental stages. The developmental stages at which cyanocobalamin supplementation

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occurred were shoot emergence, the 1-2 node stage and 3-4 node stage (Fig. 3a-c). Pea shoots supplemented with cyanocobalamin at the shoot emergence stage (Fig. 3a) accumulated less B_{12} compared with those supplemented at the two later developmental stages (Fig. 3b, c). Although there was variation in node stage present at each sampling point due to some plant developmental heterogeneity across growing trays at a commercial sowing density, the greatest B_{12} quantity always accumulated within the leaves (Fig. 3a-c). After 48 h of supplementation at all developmental stages, the leaves accumulated more than the quantity of B_{12} that is necessary to deliver the RDA of vitamin B_{12} within 15 g of plant material (Fig. 3a-c; dashed line across graph). This suggests that there is flexibility in the developmental stage at which the plants can be supplemented with cyanocobalamin to obtain a product that contains the RDA of B_{12}. This could benefit growers, who might wish to operate their horticultural facility in a flexible manner, according to staff availability and customer demand.

Senescence and simulated shelf life are unaltered by Vitamin B_{12} fortification

Senescence is a nutrient recycling process that reallocates resources within plants and can be initiated by age or stress. This causes leaf yellowing, which can reduce the shelf-life of harvested crops. We wished to determine whether our approach for the fortification of pea shoots with B_{12} affects the senescence rate of the harvested pea shoots. This is important to evaluate whether the presence of B_{12} might affect the shelf life of B_{12}-fortified salad products, relative to products that are not fortified with B_{12}. To investigate this, we used protocols that have been used previously to study the senescence of Arabidopsis leaves[32,33]. Pea shoots were cultivated as above and supplemented with cyanocobalamin at two concentrations (10 μM or 20 μM) for 48 h starting on day 4 of cultivation. After harvest on day 8, plant material was kept under conditions that simulated cold chain storage (5-6 °C in darkness) for up to 30 days (Fig. 4a). During this period, several established proxies for senescence[32] were obtained at regular intervals. These were the proportion of electrolytes leaking from plant tissue (a measure of cell

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lysis, which releases electrolytes), the chlorophyll content, and a measure of maximum photosynthetic efficiency[32]. Photosynthetic efficiency was estimated using the Fv/Fm parameter derived from chlorophyll fluorescence analysis, which provides a measure of the maximum potential quantum yield of Photosystem II whereby a lower value indicates a lower potential quantum yield. Because the photosynthetic apparatus is dismantled during senescence-induced nutrient recycling, the chlorophyll content and Fv/Fm can decrease during leaf senescence[32,34,35].

First, we monitored the B_{12} content of harvested leaf tissue that was stored under simulated cold chain conditions (Fig. 4b), to evaluate whether the B_{12} remained in the tissue during this simulated storage. Batches of tissue were sampled at several intervals during cold storage, and B_{12} content in leaves was measured using LC-MS. During this period of simulated cold chain storage, there was no significant alteration in the concentration of the biologically active form of B_{12} at either B_{12} dosing concentration tested (Fig. 4b). This indicates that a portion of pea shoots fortified with B_{12} using our approaches still contained the RDA of B_{12} after 4 weeks of simulated cold chain storage.

During dark storage of the detached leaf tissue, relative to the start of the time series, electrolyte leakage increased (Fig. 4c), chlorophyll content decreased (Fig. 4d), and Fv/Fm decreased (Fig. 4e). This is consistent with the initiation of senescence in this leaf tissue. We did not detect accelerated leaf tissue senescence following B_{12} fortification of this pea shoot variety, compared to the unfortified control, using these proxies for senescence. Over 28 days of storage under cold, dark conditions, tissue electrolyte leakage increased in the control (unfortified) tissue on days 26 and 28 post-harvest, relative to earlier time points (Fig. 4c). However, there was no consistent significant difference between plants supplied with 10 or 20 μM of cyanocobalamin, and the control, at any time point (Fig. 4c). In general, there was also no significant difference in mean abaxial chlorophyll content between the control and fortified tissue (Fig. 4d), as measured

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using a non-invasive optical method (Dualex). The decrease in chlorophyll content was retarded slightly at days 27 and 30 in pea shoots supplemented with 20 μM cyanocobalamin relative to the untreated control (Fig. 4d). Furthermore, a measure of the maximum photosynthetic efficiency of photosystem II (Fv/Fm) was generally unaltered between the control and fortified material, with the exception of timepoints 28-30 days post-harvest, where the control tissue had significantly lower photosynthetic efficiency than tissue fortified with 10 μM (Day 27) or 20 μM (Day 30) cyanocobalamin (Fig. 4e, f). Although the chlorophyll content and Fv/Fm measures could suggest slightly delayed senescence in B_{12}-fortified pea shoot tissue compared with the control, this did not occur consistently across the B_{12} concentrations tested so any effect is small. There was no obvious visible difference between the fortified and control pea shoots during simulated cold chain storage, with both undergoing some visible yellowing as tissue aged (Fig. 4g). Taken together, these data indicate that fortification of this pea shoot variety with cyanocobalamin, using the method that we developed, does not accelerate tissue senescence so cold-chain shelf life is unlikely to be altered.

Simulated digestion of B_{12}-fortified pea shoots releases bioavailable Vitamin B_{12}

Our aim was to develop a fortification strategy to deliver the RDA of B_{12}, for at least one adult, within a salad portion (15 g). We reasoned that it is important to determine whether the B_{12} accumulated within the pea shoot tissue is accessible for absorption during human digestion. Therefore, we implemented the Infogest 2.0 protocol to simulate *in vitro* the digestion in the upper gut[36]. With Infogest, it is possible to determine the quantity of a nutrient that becomes accessible for absorption at each stage of human digestion in the upper gut. The method involves the sequential incubation of the simulated meal (in this case, pea shoot tissue) with simulated digestive fluids and enzymatic mixtures that reflect the conditions found at each stage of human oral, gastric and duodenal digestion, and the release of nutrients at each of these stages (Fig. 5a)[36].

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At the start of the *in vitro* Infogest digestion analysis, the effect of chewing was simulated in two ways: by mashing or by lysing the B_{12}-fortified pea shoots. We compared the quantity of B_{12} that became accessible for absorption at the end of the simulated gastrointestinal digestion and confirmed that there was no difference between the quantity of B_{12} released using these two simulated chewing methods (Fig. 5b, c). Therefore, tissue mashing is sufficient to release B_{12} from the tissue (Fig. 5b). A meal portion (15 g) of pea shoots that was supplemented with 20 μM cyanocobalamin during cultivation released more than the RDA of B_{12} (2.4 μg) during simulated digestion (Fig. 5c).

We then compared the quantity of B_{12} released at each digestive stage, using mashed pea shoots supplemented with 10 μM or 20 μM of cyanocobalamin. This determined that the amount of B_{12} released was similar after the oral and gastric digestive phases and increased significantly when the digesta reached the duodenal phase (Fig. 5d). Pea shoots supplemented with 20 μM cyanocobalamin showed the greatest quantity of B_{12} release during the duodenal phase. Significantly more B_{12} was released from pea shoots supplemented with 20 μM cyanocobalamin compared with 10 μM (Fig. 5d; duodenal phase). Overall, these experiments identified that B_{12} was released during simulated digestion of pea shoots fortified with B_{12}, using the strategy that we developed, and this B_{12} would become available for digestive absorption.

Economic analysis

We conducted a simple techno-economic analysis (TEA) to examine how variation in cyanocobalamin sourcing costs and fortified nutrient solution reuse influences commercial feasibility in an aeroponic farm. The economics described here reflect those at the time of writing (August 2025). B_{12} is traded internationally and produced predominantly in China, which accounts for the majority of global supply[37-39]. Our techno-economic analysis was not designed to model the full cost of pea shoot production, but instead to isolate additional costs arising from B_{12} fortification using our aeroponic approach. Baseline production costs relevant to any

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commercially-produced pea shoots, such as seed, land, capital equipment and operational labour were not modelled, since they vary widely by growing system and region.

The TEA considered three input categories: the chemical cost of cyanocobalamin across sourcing tiers, specific energy costs of atomizers used for aeroponic mist generation, and labour specifically attributable to dosing the nutrient reservoir with cyanocobalamin. Labour and atomizer running costs were considered fixed, and three different price tiers for cyanocobalamin were used: wholesale food-grade, an active pharmaceutical ingredient (API) benchmark, and a pharmaceutical-grade laboratory-reagent. Cyanocobalamin fortification concentrations of 10 μM and 20 μM were evaluated**,** because our data show these concentrations are sufficient to fortify a single portion of pea shoots (15 g) to the RDA.

Our baseline scenario assumes no reuse of cyanocobalamin-supplemented nutrient solution. With this, the additional cost of fortifying 1 kg of pea shoots to the B_{12} RDA using 10 μM cyanocobalamin was **~**£1.35/kg (wholesale), £1.56/kg (API), and £89–90/kg (laboratory reagent); and for 20 μM cyanocobalamin, **~**£1.44/kg (wholesale)**,** £1.67/kg (API)**,** and £178–180/kg (laboratory reagent). On a per-portion basis (15 g pea shoots), this corresponds to an additional cost of ~£0.02–£0.023 (10 μM cyanocobalamin) and ~£0.022–£0.025 at (20 μM cyanocobalamin) when sourced from bulk or API supply chains, which we assume would be used commercially (Supplementary Fig. 2).

Because hydroponic/aeroponic systems often recirculate the nutrient solution, we also modelled the cost reduction through nutrient solution reuse, whilst estimating cyanocobalamin loss scenarios due to plant uptake and environmentally-induced degradation. Although direct measurements of cyanocobalamin depletion in recirculating systems are not available, studies of hydroponic operation have shown that nutrient recycling leads to nutrient depletion[40,41]. Cyanocobalamin is also sensitive to light-induced degradation in aqueous solution[30]. To reflect these combined risks, we applied progressive accumulative losses with reuse cycles in our

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model of 5% loss for two reuse cycles up to 25% loss for 10 reuse cycles. Despite estimated cyanocobalamin degradation, reuse markedly reduced fortification costs. After 10 reuse cycles, incremental costs for wholesale-sourced B_{12} fell to ~£0.61/kg at 10 µM cyanocobalamin and ~£0.66/kg at 20 µM cyanocobalamin, equivalent to ~£0.009–0.010 per 15 g portion, representing a ~55% cost reduction compared with no nutrient solution reuse. Similarly, for API-sourced B_{12}, costs reduced by 57% to ~£0.67/kg (10 µM) and ~£0.72/kg (20 µM), or ~£0.010 - £0.011 per 15g portion. Therefore, while cyanocobalamin price dominates the economics, solution recycling could deliver large proportional savings. In the commercially relevant wholesale and API cyanocobalamin cost scenarios, recycling could reduce costs to below £0.01 per portion. These savings would scale significantly when applied to commercial production volumes.

Discussion

Here, we developed an approach leading to the fortification of a single portion of pea shoots with the RDA of Vitamin B_{12}. The approach functions effectively within a scalable commercial growing environment. We envisage that this could be used to produce a component for addition to packaged salads, to increase the options by which people can supplement their diet with B_{12}. Because the method allows the delivery of B_{12} within a meal, rather than a tablet, we reason that its uptake during digestion will be optimised because food consumption will lead to production of factors (haptocorrin, intrinsic factor) required for B_{12} absorption. The approach does not alter the rate of senescence of harvested plant tissue, so seems unlikely to alter product shelf life. Given that the B_{12} persists within the plant tissue for at least 4 weeks (Fig. 4b), the material could- in theory- be distributed to most locations globally if the economic case existed.

While we selected pea shoots for this work, it is possible that the approach could be adapted to other salad crops to increase its versatility. Previous studies reported that hydroponics can be

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used to fortify certain additional species with B_{12} (Supplementary Table 1), suggesting this could be adapted to aeroponics. However, approaches with additional species require work to determine whether the B_{12} is bioaccessible during digestion, is accumulated in forms that are active in humans, and to evaluate commercially viability. Through a direct comparison, we found that the approach we developed was several orders of magnitude more effective than a method described by a patent[27] that involves soaking pea seeds in B_{12} solution (Fig. 2b). This is important because commercially available B_{12} is expensive, so a B_{12} accumulation level within plant tissue that meets commercial objectives is crucial for deployment at scale. We also conducted a small-scale comparison with hydroponic cultivation, which indicated that this can be used to fortify pea shoots with B_{12} (Supplementary Table 1), albeit requiring a larger nutrient tank volume and hence greater B_{12} input cost. It is possible that aeroponic cultivation could be used to deliver other vitamins or minerals into salads in a relatively straightforward manner, although it would require experimental optimization and validation for any combination of nutrient and crop. Furthermore, we reason that the method could be combined with other fortification methods, such genetic approaches to increase iron accumulation within plant tissue, to fortify salads in a combinatorial manner. An alternative approach for salad fortification with B_{12} has been proposed that involves co-culture and colonisation of plants with bacteria that over-produce B_{12}[42]. An advantage of this approach is that it eliminates the need to source and purchase food-grade cyanocobalamin, whereas the microorganisms may present difficulties with food safety regulators. This approach could combine well with indoor farming, although challenges with bacterial overgrowth, biofilm formation in the irrigation systems, and sterilization of the nutrient solution would need to be overcome.

Although our experiments used a legume, root nodules did not form because the relatively clean growing environment means that nod factor was likely absent (Fig. 1b) and the plants were grown for a short duration. However, the presence of microorganisms in certain soil types might

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present an alternative approach to fortify crops with B_{12}. For example, B_{12} content of spinach was elevated when cultivated on organic soils containing farm animal manure[22], presumably due to microbially derived B_{12}. Other soil bacteria such as strains of *Pseudomonas fluorescens* can produce B_{12}[43], and the production of B_{12} by rhizobia including *Sinorhizobium meliloti* opens future possibilities for legume fortification by B_{12} derived directly from naturally associated bacteria[44]. As the strategy that we describe uses indoor farming technology that has increased energy requirements and requires capital expenditure, it might not be suitable for B_{12} fortification in all regions globally, so approaches such as fortification using B_{12}-producing bacteria[42] could offer a less resource-intensive alternative. A further way that plant-based foods might be supplemented with B_{12} is through fermentation, although it is unclear whether the B_{12} present in foods such as natto contributes to serum B_{12} levels[45].

Use of aeroponic cultivation could, in principle, improve B_{12} uptake into the plant perhaps by allowing greater root respiration, or by altering root development or size[29] such that greater B_{12} quantities can enter the plant. Importantly, aeroponic cultivation allows the use of a smaller volume of nutrient solution compared with hydroponics, thus reducing the overall quantity of expensive B_{12} that must be added to the nutrient mix. Specifically, the reduced volume of B_{12} solution that is needed during aeroponic cultivation combined with aerosolization increases the probability that each root interacts with B_{12} (compared with a larger volume of solution that would be required by hydroponics). Growers might wish to recapture the B_{12} solution and use it again after root exposure to B_{12}. One challenge with recapture and reuse that will need to be overcome is to avoid cyanocobalamin degradation by the UV light sources used to sterilize the nutrient mix in indoor growing environments.

The mechanism(s) of B_{12} uptake by the pea shoots are unknown, and could be symplastic or apoplastic. Accumulation of B_{12} in the leaves could occur through the action of the transpiration stream, given that cyanocobalamin is water soluble. If this is the case, it might be possible to

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increase B_{12} accumulation in the leaves through environmental treatments that maximise stomatal aperture during the B_{12} treatment period, or that take advantage of the times of day of maximum stomatal aperture. The accumulation of B_{12} in the roots could occur due to a restriction of uptake by the Casparian strip, but understanding this will require detailed knowledge of the uptake pathway. Greater B_{12} accumulation after the cotyledon stage (Fig. 3) could simply reflect a greater tissue volume to store B_{12} in larger plants, or developmental stage-specific changes in vascular configuration or root anatomy. Although identification of the uptake mechanism might allow for breeding of crop varieties that have greater efficiency of B_{12} accumulation, this does not appear necessary in the case of the pea shoot that we tested because it was straightforward to obtain the RDA of B_{12} within the leaf tissue of an existing commercial pea shoot variety. In the future, it will be useful to obtain more information about the uptake mechanism and location of B_{12} storage within the plant tissue, since this could be important for extending the number of crop species to which the approach can be applied. It will also be informative to understand how different horticultural nutrient formulations affect B_{12} uptake. A previous study using hydroponics and *Glycine max* (soybean) found that B_{12} uptake was not impacted by a respiration inhibitor[24], suggesting that it is not an active process in soybean, but it is unknown whether this generalizes to other crops and growing conditions. If uptake is an active process that involves membrane transport mechanisms, we speculate that it might be possible to engineer active B_{12} uptake from the growing media.

The approach that we developed used a commercial indoor farming environment. This overcomes several limitations that would arise from attempting B_{12} fortification in outdoor environments. First, B_{12} is degraded by the UV wavelengths present in sunlight[46], whereas indoor growing environments use either LED light sources that generally lack UV wavelengths, or cultivation occurs under glass that filters UV from sunlight. Second, B_{12} is degraded by various microorganisms (including soil bacteria)[47,48], so using a relatively clean indoor growing

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environment reduces the likelihood of B_{12} loss by microbial activity. The method could be adapted for greenhouse cultivation, particularly when combined with aeroponic irrigation, but might need optimization of the light, temperature and humidity conditions that lead to the greatest efficiency of B_{12} accumulation within the harvested tissue.

Our TEA suggests that aeroponic fortification pea shoots with B_{12} is commercially viable, with minimal cost implications when wholesale or API supply chains are used for cyanocobalamin. Importantly, this TEA did not attempt to capture the full cost structure of fortified pea shoot production – which varies widely with many operational costs such as seeds, infrastructure, and operational labour. Instead, we wished to estimate the additional cost introduced by B_{12}-fortification under our aeroponic dosing method. Our analysis also predicts that nutrient solution reuse will increase cost efficiency. Cyanocobalamin degradation will depend on local environmental conditions, so cyanocobalamin loss coefficients during reuse may require customization to individual commercial growing environments.

Conclusion

We have developed a straightforward and commercially viable approach for the fortification of a salad crop with a quantity of Vitamin B_{12} that exceeds the RDA. Given that indoor farms are amenable to interventions that raise the nutritional value of horticultural products[49], B_{12} fortification could be stacked with other types of fortification to further enhance the nutritional content and commercial value of the product. Focusing on multiple aspects of B_{12} fortification in a single species has enabled us providing a roadmap for this type of crop fortification in other species and with other nutrients, from the underpinning biology to an economic evaluation. The approach described here represents one strategy for combating B_{12} insufficiency or deficiency, and has the capacity for commercial deployment for production at scale.

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Methods

Plant material and growth conditions

We selected a commercial variety of *Pisum sativum* (CN Seeds cultivar 4019) for this work. Cultivar 4019 is suitable for indoor farm production because it grows rapidly and lacks tendrils, providing greater compatibility with automated processing and packing equipment. In all cases, the pea shoots were cultivated in an experimental aeroponic indoor farm (LettUs Grow, Bristol, UK) that uses commercially available equipment (Supplementary Fig. 3a). The cultivation system used an aero-hydro cultivation method[29], which has undermounted ultrasonic devices that produce a nutrient mist at regular intervals from the surface of a thin layer of water at the base of the growing bench[50]. This produced an aerosol with particle sizes ranging 5-15 μm at the point of landing. In this vertical farming system, each cultivation stack comprised 4 layers of growing shelves and lighting (Supplementary Fig. 3b).

To induce germination, dried pea seeds were imbibed in cold water for two days with gentle agitation. The seeds were pre-rinsed to clean surface debris, and then transferred to the imbibition tank. During imbibition, agitation occurred through the action of a pump that supplied aerated water to the imbibition system for 15 minutes during each hour. This water was refreshed at least once per day. Imbibed peas were subsequently transferred to jute horticultural matting that was pre-saturated with water and placed on horticultural trays. Imbibed peas were sown at a density of 357 g per horticultural tray. These were transferred to a dark germination room that was maintained at 26 °C and 90% humidity, for 3 days. At this point, cotyledons had emerged, and radicles were sufficiently long to pass through the jute matting. After this, horticultural trays were transferred to the aeroponic cultivation environment (grow beds), with each aeroponic bed holding four trays. Aeroponic mist generation was operated with a duty cycle of 1 minute misting on, 4 minutes misting off. Pea shoots were cultivated in the aeroponic system for 8 days to the 3-4 node stage, and for shorter durations for comparisons of

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developmental stages (Fig. 3). For all experiments, the growth medium (nutrient mix) was VitaLink HydroMax nutrient A+B mixes (Supplementary Table 2) (HydroGarden, Coventry, UK), maintained at pH 6.2 and an electrical conductivity of 1.7 μS cm^{-1}. Growing conditions comprised a 16 h photoperiod with 24 °C day temperature, 17 °C night temperature, and 70-75% relative humidity). Lighting was provided with Valoya AP673L horticultural LED sets (Valoya, Helsinki, Finland; plant height PAR of 150 μmoles photons m^{-2} s^{-1}). The nutrient solution temperature was 17 °C. Chamber CO_2 levels were approximately 800 ppm. The cultivation bed volume was 20 L and reservoir volume was 1000 L. In addition to automated farm monitoring, during experimentation the nutrient mix pH was monitored using a Hanna Instruments HI-98128 Pocket pHep5 Water resistant pH tester (Hanna Instruments, Rhode Island, USA), and electrical conductivity and grow bed temperature measured using a Hanna Instruments HI-991300 electrical conductivity (Hanna Instruments, Rhode Island, USA), total dissolved solids, and temperature meter.

Aeroponic Vitamin B_{12} supplementation methods

The B_{12} analogue cyanocobalamin was used for all experiments. Cyanocobalamin (Merck, Darmstadt, Germany) was prepared as 1 mM stocks in H_2O up to a week before each experiment. Cyanocobalamin stocks were stored at 4 °C in light-excluding conditions to ensure stability of the molecule. Cyanocobalamin was added at concentrations of 10 – 40 μM, with the quantity of stock solution added calculated according to the total volume of nutrient solution in the growing system. Cyanocobalamin was added only once to the growing system, we did not monitor or top up its concentration, and its addition was conducted manually. Cyanocobalamin can degrade outside a pH range of 4-6.5 and in the presence of UV light[51] (one study indicates it has greatest stability at pH 6.3[52]). Therefore, for the duration of the cyanocobalamin treatment of the plants, the pH was carefully controlled (pH 6.2, as above) and the nutrient mix was disconnected from the reservoir so that it was not exposed to a sterilizing UV treatment. During

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experiments that replicated a patent[27] where B_{12} was added during seed imbibition, the cyanocobalamin was added at the required concentration to the seed imbibition tank, prior to germination.

Tissue sampling

For all experiments, sampling of pea shoot tissue started at dawn, following an aeroponic cultivation period of either 8 days (Fig. 2, Fig. 4, Fig. 5) or shorter periods (Fig. 3). Tissue was harvested on a per horticultural tray basis, with pea shoots cut to the base of the jute matting using a single-cut method. When needed for B_{12} measurements (Fig. 2), roots were cut from underneath the jute mat. The fresh weight of tissue was measured at the time of sampling and, in the case of roots, excess water was carefully squeezed from the roots before weighing (this was necessary because aeroponic irrigation leads to considerable amounts of water within the roots). For analytical purposes, tissue was obtained from five locations within each cultivation tray to obtain representative information about B_{12} accumulation across the growing tray (Supplementary Fig. 3c). Tissue from the five locations on each tray was pooled, and comprised one replicate for analytical purposes. A minimum of 4 replicate trays were sampled per treatment (specified in figure legends). Sampling was conducted by 3-4 people working as a team, with team members blind to the cyanocobalamin treatments. Tissue was placed into 50 ml Falcon tubes and frozen in dry ice prior to longer-term storage at -80 °C before analysis.

Vitamin B_{12} extraction and quantification

For quantification of B_{12} content, a minimum of 50 mg of frozen tissue was lysed using a TissueRuptor II (Qiagen, Hilden, Germany). 5 ml of extraction buffer (50 mM HEPES, 200 mM NaCl, 1 mM DTT, 1 % (v/v) Triton-X 100, pH 7.8) and an internal standard was added to the sample prior to vortexing for 5 min. The samples were incubated at 80 °C for 40 min to release the bound B_{12}, then centrifuged. The supernatant was removed, and retained. The pellet was lysed again with a TissueRuptor II and a further 5 ml of extraction buffer added, before vortexing

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and centrifugation, to ensure all bound vitamin B_{12} was released from the sample into the supernatant. The supernatants were pooled, and 1 ml of this was filtered on a spin column to remove any remaining debris from the sample for analysis.

20 µl of sample was injected for LC-MS analysis of B_{12} content. The vitamin B_{12} concentration was resolved on a UPLC HSS T3 1.8 µM column with 0.1% (v/v) formic acid in water, and 0.1% (v/v) formic acid in acetonitrile with a gradient flow for 20 minutes on an LC-MS instrument (Agilent 1290 infinity II; Agilent, California, USA). Through multiple reaction monitoring, the vitamin B_{12}-specific ion 678-147 was detected and quantified. The B_{12} was quantified using matrix match calibration ranging (0-100 ng ml^{-1}) using a vitamin B_{12}-free control tissue sample.

Senescence experiments

For all measures of senescence (electrolyte leakage, chlorophyll content, chlorophyll fluorescence; Fig. 4), pea shoots were cultivated for 8 days, to the 3-4 node stage. The aerial tissue was harvested as described previously, and stored in sealed bags, in darkness, at 4 °C, to simulate cold chain storage. During this period of storage senescence measures were obtained at regular intervals (Fig. 4b-g). Electrolyte leakage is a destructive measure, whereas measurements of chlorophyll content and chlorophyll fluorescence are not destructive.

Electrolyte leakage

Electrolyte leakage due to cell degradation during senescence was measured using an established method[53]. For each timepoint, separate batches of tissue were used for analysis to avoid re-sampling the same leaves. For each replicate at each timepoint, three 5 mm discs were cut from separate leaves of an individual plant using a cork borer, and placed in a glass test tube that was pre-washed with MilliQ water. 5 ml of MilliQ water was applied to the leaf discs, and the tubes were shaken at 120 rpm for 3 h at room temperature. The water was removed, and its electrical conductivity was measured (EC_{pre}). To release all electrolytes, leaf discs were

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transferred to a clean tube, and frozen to -70 °C for 1 h and then allowed to thaw gradually[53-55]. This ruptured the tissue and release all remaining cellular electrolytes. The 5 ml of water removed previously was returned to the tube, which was shaken at 120 rpm for 3 h at room temperature. Subsequently, the electrical conductivity of the water was measured again (EC_{frz}). The proportion of total electrolytes leaking from the tissue was expressed as [EC_{pre} / EC_{frz}] * 100.

Chlorophyll content

Chlorophyll levels were estimated using a non-destructive optical method (Dualex 4, Force-A Scientific, Paris, France). Briefly, the instrument was clamped onto replicate leaves and measurements obtained according to the manufacturer's instructions. The Dualex instrument measures the ratio of light transmitted through the leaf at 710 nm and 850 nm, and calculates the chlorophyll content according to the manufacturer's research[56]. For each replicate plant, the mean of 3 or 4 measurements was obtained from the abaxial surface of each leaf.

Chlorophyll fluorescence

Because plants were small and fragile, we used chlorophyll fluorescence imaging rather than an approach using a leaf clip and fibre optic. The ratio of variable chlorophyll fluorescence to maximum chlorophyll fluorescence (F_v/F_m) was measured using an Imaging-PAM M series (MAXI Version, Walz, Effeltrich, Germany). Prior to use, the aperture and gain settings were adjusted to ensure that the maximum fluorescence signal was between 1.5 and 2 (arbitrary units), to provide an appropriate dynamic range. Leaves were dark adapted for 20 minutes prior to measurement by placing the plant material in a light-excluding container, and the F_0 (minimum fluorescence level in the dark-adapted state) and F_m (maximum fluorescence yield following a saturating light pulse) parameters were obtained. A saturating pulse of instrument intensity setting level 2 was used[57]. F_v/F_m was calculated as $(F_m-F_0)/F_m$. Data were processed initially using ImagingWIN software (Walz), which was also used to control the imaging system.

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Each leaf was considered to be one individual replicate, with the mean fluorescence signal obtained across the entire leaf surface and all leaves on the plant measured.

Comparison with hydroponics

P. sativum seeds were germinated and grown in compost at for 8 days, at room temperature. Seedlings were selected randomly for subsequent experimentation. After 8 days growth, excess soil was removed from roots and plants were cultivated hydroponically for 2 days without cyanocobalamin supplementation. Roots were then submerged 10 μM cyanocobalamin for 48 h. Tissue above the second node was detached, frozen at -80°C, and ground in liquid N_2 before storage at -80°C prior to B_{12} extraction and analysis[58,59].

INFOGEST simulation of digestive B_{12} release

For analysis of digestive B_{12} bioaccessibility (Fig. 5), pea shoots were cultivated for 8 days, to the 3-4 node stage. Tissue was in subsequent analyses within two days of harvest. Vitamin B_{12} bioaccessibility in pea shoot leaves was assessed by simulating their digestion *in vitro* in the upper gastro-intestinal tract, following the Infogest 2.0 static method[36]. Infogest has been used successfully to measure digestive accessibility of vitamin B_{12}[60,61]. Digestions were conducted in triplicate with a simultaneous control digestion run, within which water replaced the pea shoots to account for background signal arising from the added fluids, enzymes, and bile salts. Enzyme activities and bile salt concentrations were calculated beforehand. Pepsin from the porcine gastric mucosa (Sigma P7012); pancreatin from porcine pancreas (Sigma P7545); bovine bile (Sigma B3883) and salts used in the preparation of the simulated digestion fluids (KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$, $(NH_4)_2CO_3$, HCl and $CaCl_2$ $(H_2O)_2$) were obtained from Sigma-Merck.

In brief, a meal consisting of 0.4 g of either smashed or lysed pea shoot leaves with 0.6 ml of water was mixed at 40 rpm using an orbital shaker, at 37 °C. To this mixture, 0.8 ml of simulated

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salivary fluid, 5 µl of 0.3 M $CaCl_2$, and 0.195 ml of water were added at pH 7 for the oral phase. After 2 minutes, the gastric phase was initiated by adding 1.6 ml of simulated gastric fluid, reducing the pH to 3 with 1M HCl, followed by addition of 1 µl 0.3 M $CaCl_2$, 0.264 ml water, and 0.1 ml of a pepsin solution to achieve 2000 units of pepsin per ml in the final gastric digestion mixture. The duodenal phase commenced 1 hour later by adding 1.7 ml of simulated intestinal fluid to the gastric chyme, increasing the pH to 7. Then, 8 µl of 0.3M $CaCl_2$, 0.792 ml water, 0.5 ml of bile solution (yielding a final concentration of bile salts of 10 mM), and 1 ml of pancreatin (to achieve 100 units of trypsin per ml of final digestion mixture) were added to the digestion mixture. The duration of the duodenal phase was 3 hours, after which the digested samples were boiled at 99 °C for 5 minutes to inactivate the enzymes. The digested mixture was then centrifuged at 4500g for 10 minutes to separate the released nutrients in the supernatant from the pellet. Both supernatant and pellet were subsequently frozen for storage before being analysed.

Techno-economic analysis

The techno-economic analysis (TEA) was designed to identify additional costs of B_{12} fortification of pea shoots in aeroponic horticulture, rather than model the full economics of pea shoot production. This is because the latter will vary substantially by growing platform, geography, and local markets. We specifically considered the additional inputs that are required for our fortification approach: (1) chemical costs of cyanocobalamin across sourcing tiers, (2) energy use costs of aeroponic atomizers in our mist-based B_{12}-fortification method, (3) additional labour time for dosing with cyanocobalamin. Costs of pea shoot production such as seed, land, and baseline energy requirements of the production process were excluded, since they are growth system and region-specific. Our aim was to estimate the additional costs arising from B_{12}-fortification of a kg or portion of fortified pea shoots.

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We modelled additional costs of pea shoot Vitamin B_{12} fortification at 10 μM and 20 μM cyanocobalamin supplementation in an aeroponic system, in a glasshouse with natural lighting. In the model, growing beds each contained 20 L of nutrient solution and produced 4 kg of pea shoots per growth cycle, i.e. 5 L kg^{-1} nutrient solution application volume. The energy cost for ultrasonic atomisers was fixed at £0.432/kg per cycle, and cyanocobalamin dosing labour was modelled at £0.05/kg based on a farm operator spending one minute dosing B_{12} into each aeroponic bed. Labour cost was benchmarked to the UK National Living Wage of £12.21/hr (UK Government, 2025).

Cyanocobalamin costs were parameterised using three sourcing tiers: wholesale food grade (£1.30/g; Made-in-China (aggregates supplier quotes from major Chinese manufacturers), accessed August 2025), active pharmaceutical ingredient (API) (£1.56/g; PharmaCompass, accessed August 2025), and laboratory grade reagent (£89.1/g; Thermo Fisher, accessed August 2025). Because wholesale and API prices for B_{12} are not reported systematically in the scientific literature, we drew from representative commercial listing sources commonly used in techno-economic analysis. API benchmarks were obtained from PharmaCompass, an industry platform that compiles manufacturer and trader data. Pharmaceutical-grade laboratory reagent prices were from the ThermoFisher Scientific catalogue. These sources were used to provide realistic input assumptions for cost modelling, rather than to serve as definitive price references.

Loss fractions for nutrient recycling were informed by evidence of micronutrient depletion in recirculating hydroponics[40,41] and stability studies of cyanocobalamin in aqueous systems[30]. To capture plausible degradation from microbial metabolism, precipitation, filter-induced light exposure, and solution handling, cumulative losses were conservatively assumed at 5%, 10%, 15%, 20%, and 25% at 2, 4, 6, 8, and 10 reuse cycles, respectively. To achieve the target dosing concentrations in a 20 L aeroponic bed, supplementation from a 1 M cyanocobalamin

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stock would be 0.2 mL (10 μM) and 0.4 mL (20 μM). These values were used as a dilution check to confirm the consistency of cost calculations.

The following equations were used to estimate the additional cost of fortifying pea shoots with vitamin B_{12} using dosing concentrations of 10 or 20 μM, with and without nutrient solution reuse. To calculate the cost of cyanocobalamin per litre of nutrient solution at a given dosing concentration of cyanocobalamin (Equation 1):

$$C_l = (C_g\ M\ d)\ \mathrm{x}\ 10^{-6}$$

Where C_l is the cost of cyanocobalamin per litre, C_g is the per gram cost of cyanocobalamin, M is the molecular weight of cyanocobalamin (1355.4 g mol^{-1}) and d is the desired dosing concentration in μM (typically, 10 or 20 μM).

To calculate the cost of cyanocobalamin per kg of pea shoots produced, a simple multiplication was used because 5 litres of nutrient solution are required to produce 1 kg of pea shoots (Equation 2):

$$C_{kg} = 5C_l$$

Where C_{kg} is the cost of cyanocobalamin per kg of pea shoots, C_l is the cost of cyanocobalamin per litre of nutrient solution used from Equation 1, and 5 litres of nutrient solution are required to produce 1 kg of pea shoots.

When considering the reuse of nutrient solution, the reuse-adjusted cyanocobalamin cost per kg of pea shoots produced accounting for nutrient losses was (Equation 3):

$$C_{ra} = C_{kg}\left(\frac{N-(N-1)r}{N}\right)\frac{1}{1-L_N}$$

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Where C_{ra} is the reuse adjusted cost of the nutrient solution, C_{kg} is the cost of cyanocobalamin per kg of pea shoots (Equation 2), N is the number of solution reuse cycles (e.g. 2, 4, 6, 8), r is the fraction of the nutrient solution that is reused (1 in this model, representing complete reuse), and L_N is the cumulative cyanocobalamin loss fraction after N reuse cycles (assumed as 0.05, 0.10, 0.15, 0.20 or 0.25, respectively).

The total additional cost of fortifying pea shoots with cyanocobalamin, per kg, was calculated as (Equation 4):

$$C_f = C_{ra} + 0.432 + 0.05$$

Where C_f represents the total additional cost of fortification per kg, Cra is the reuse adjusted cost of cyanocobalamin within the nutrient solution (Equation 3), £0.432 represents the fixed energy cost of ultrasonic atomisation per kg of pea shoots, and £0.05 represents the estimated labour cost.

Statistics and reproducibility

Details of replication levels and specific statistical analyses used are provided in the figure legends. The TEA models derive from simulations, thus lack statistical analyses. Sample sizes: Fig. 2a (n = 6), Fig. 2c, d (n = 4, except Fig. 12c where n = 2), Fig 2e (n = 4-12), Fig. 3a-c (n = 4), Fig. 4b (n = 5 (week 1), n = 3 (week 2), n = 3 (week 3), n = 4 (week 4), Fig. 4c (n = 13), Fig. 4d (n = 14), Fig. 4e, f (n = 14), Fig. 5b-d (n = 3), Supplementary Fig. 1a-l (n = 14). All statistical analyses and data presentation used R v4.5.1 (https://www.r-project.org). R packages used for analysis and figure preparation were ggplot2, scales, reshape2, cowplot, gridExtra, ggthemes, ggpubr, tidyverse, dplyr, multcompView and rstatix. All underlying source data for main figures are in Supplementary Data 1.

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Figure legends

Figure 1. Approach for the fortification of pea shoots with Vitamin B_{12}. (a) Schematic of the process of fortification with B_{12} during aeroponic horticulture. (b) Appearance of growing bed that is supplemented with cyanocobalamin. Pea plant roots are protruding from underside of jute matting, and red colour in aeroponic bed is caused by presence of cyanocobalamin. Created in BioRender. Dodd, A. (2026) https://BioRender.com/scq4r3p.

Figure 2. Effective fortification of pea shoots with Vitamin B_{12} during aeroponic cultivation. (a) Comparison of the quantity of cyanocobalamin accumulated within pea plant leaves after cultivation with aeroponics, and an alternative method involving the soaking of seeds in cyanocobalamin during imbibition (n = 6; Statistical significance was determined using two-way ANOVA and Tukey HSD *post hoc*, with different letters indicating significant differences at $p \leq 0.05$). (b) Comparison of mass of pea shoots that must be harvested to obtain plant tissue that contains the USA RDA of B_{12} (2.4 µg), when pea shoots were fortified using two different methods. Note the differing y axis scales on the graphs for the two methods. (c, d) In two independent experiments, comparison of the quantity of cyanocobalamin present within several parts of the plants (n = 4, except for seed material in 1c, which has n = 2; statistical significance was determined using two-way ANOVA and Tukey HSD *post hoc*, with different letters indicating significant differences at $p \leq 0.05$). Pea shoots were harvested after 8 days of growth (3-4 node stage). (e) Across five independent trials, comparison of B_{12} accumulation within pea shoot leaf tissue after 8 days of growth (3-4 node stage). Data are calculated on a per 15 g fresh weight basis (equivalent to one portion). Horizontal dashed line indicates the USA RDA of B_{12} (2.4 µg). Statistical significance was determined using one-way ANOVA and Tukey HSD *post hoc*, with different letters indicating significant differences at $p \leq 0.05$; n = 4 - 12). In box plots, the box indicates the interquartile zone with the median line at the centre, whiskers indicate interquartile range, a black dot indicates the mean, and black lines connected to the dot indicate the s.e.m.

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Figure 3. Flexibility in developmental stage at which pea shoots accumulate the RDA of B_{12}. Representative diagrams and B_{12} content of 15 g (equivalent to one portion) of three parts of pea shoot plants, when supplemented with B_{12} for 48 h at (a) shoot emergence stage, (b) 1-2 node stage and (c) 3-4 node stage (n = 4). Dashed horizontal line on graphs indicates 2.4 µg of B_{12}, which is the US RDA. Statistical significance was determined using two-way ANOVA and Tukey HSD post hoc analysis, with different letters indicating significant differences at $p \leq 0.05$). In box plots, the box indicates the interquartile zone with the median line at the centre, whiskers indicate interquartile range, a black dot indicates the mean, and black lines connected to the dot indicate the s.e.m. Parts created in BioRender. Dodd, A. (2026) https://BioRender.com/hhdlx3o.

Figure 4. Pea shoot fortification with B_{12} does not accelerate senescence of harvested plant tissue during simulated cold-chain storage. (a) Experimental workflow for investigation of post-harvest senescence after B_{12} fortification. (b) During simulated cold-chain storage, comparison of B_{12} content of harvested pea shoots previously supplemented with 10 µM and 20 µM during each week of storage (n = 5 (week 1), n = 3 (week 2), n = 3 (week 3), n = 4 (week 4), black bar = ± SEM. Statistical comparisons were performed within each B_{12} treatment concentration, using one-way ANOVA and Tukey HSD *post hoc*, with different letters indicating significant differences at $p \leq 0.05$). (c) Proportion of total electrolytes released from pea shoots supplemented with several concentrations of cyanocobalamin, compared with no treatment control, during 28 days of simulated cold chain storage. Measurements on successive days were from separate plants, to avoid resampling the same plant material (n = 13, shaded ribbons = ± SEM; no comparisons of the control against cyanocobalamin-treated plants were significant at $p \leq 0.05$). (d) Mean abaxial chlorophyll content of pea shoots supplemented with several concentrations of cyanocobalamin, compared with no treatment control, during 28 days of simulated cold chain storage. Chlorophyll content was estimated using an optical method (Dualex instrument) (n = 14, shaded ribbons = ± SEM; statistical comparisons are of

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cyanocobalamin-treated plants against untreated control). (e, f) Maximum efficiency of photosystem II (PSII) (Fv/Fm) of pea shoots, in plants supplemented with several concentrations of cyanocobalamin, compared with no treatment control, across 28 days of simulated cold chain storage (n = 14; shaded coloured ribbons = ± SEM; statistical comparisons are of cyanocobalamin-treated plants against untreated control). (f) Representative images of Fv/Fm signal from pairs of pea shoot leaves during simulated cold chain storage. Images for each day of dark incubation are from different plants. (g) Visual appearance of representative pea shoots supplemented with 10 μM cyanocobalamin, with no treatment control, over 30 days of simulated cold chain storage. Images for each day are from different plants. Both treatments underwent a slight yellowing towards the end of the time-series. In c-e, asterisks indicate independent pairwise t-test comparisons of each treatment timepoint with the control (0 μM cyanocobalamin), where * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ (corrected for multiple testing using Bonferroni method); asterisks above or below lines indicate the B_{12} concentration is significantly greater or lower than the control, respectively. Panel (a) created in BioRender. Dodd, A. (2026) https://BioRender.com/e463jgk.

Figure 5. INFOGEST analysis establishes that B_{12} incorporated into pea shoots becomes accessible for absorption during simulated digestion. (a) General INFOGEST workflow, showing steps involved in the measurement of bioaccessible B_{12} at the end of the simulated oral, gastric and duodenal digestive phases. All assays were combined with enzymatic controls to eliminate any interference by the simulated digestive fluids or enzymes upon the measurement of vitamin B_{12}. (b) Proportion of B_{12} released by simulated gastrointestinal digestion of pea shoots supplemented with two concentrations of cyanocobalamin. Pea shoots were either lysed (as for previous experiments) or mashed, to simulate chewing. (c) Quantity of B_{12} released by simulated gastrointestinal digestion of pea shoots that was either mashed or lysed. Data are presented according to the quantity of B_{12} that would be released from a 15 g portion of pea

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shoots, showing the 2.4 µg RDA for B_{12} as a horizontal dashed line. (d) After supplementation with two concentrations of cyanocobalamin, the quantity of B_{12} released from mashed pea shoot tissue within three digestive phases. Data are presented according to the quantity of B_{12} that would be released from a 15 g portion of pea shoots, indicating the 2.4 µg RDA for B_{12} as a horizontal dashed line. (b-d) n = 3; in box plots, the box indicates the interquartile zone with the median line at the centre, whiskers indicate interquartile range, a black dot indicates the mean, and black lines connected to the dot indicate the s.e.m. Statistical significance was determined using two-way ANOVA and Tukey HSD *post hoc* test, and significant differences at $p \leq 0.05$ are indicated with different letters.

Competing interests: Authors JT, AW, LRM and JF are employees of LettUs Grow, which designs and manufactures indoor farming technology. None of the authors in academic institutions have commercial or personal financial interests in LettUs Grow.

Data availability: Source data are included within the Supplementary Information of this study. Data for the graphs in the main figures are within Supplementary Data 1. All other data are available on reasonable request from the corresponding author.

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Author contributions

BME, SGJ, NPM, JS, SS, DAL, LRM, TH, SEC, JT and AW designed and conducted experimentation. BME, NPM, JS, LLBD analyzed and presented data. BME, NPM, JS, LLBD, NR, KAF, CE, JC, JF, MW and AND interpreted data and wrote the manuscript. BME, KAF, CE, JC, JF, MW and AND conceived the project, obtained funding, and supervised the project.

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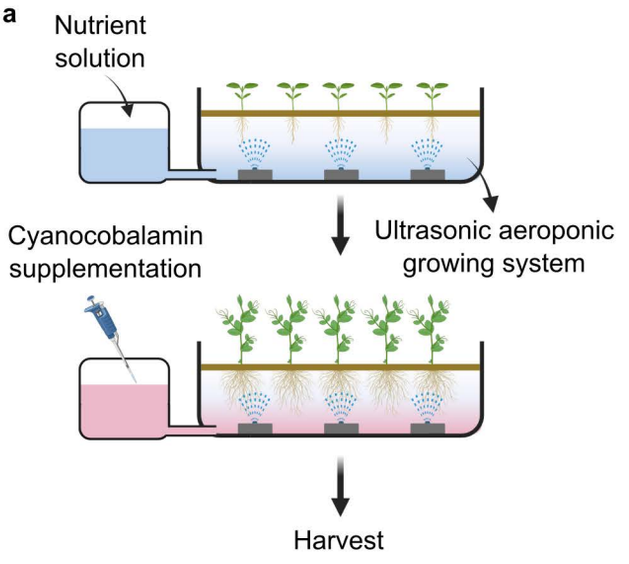
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Editorial Summary

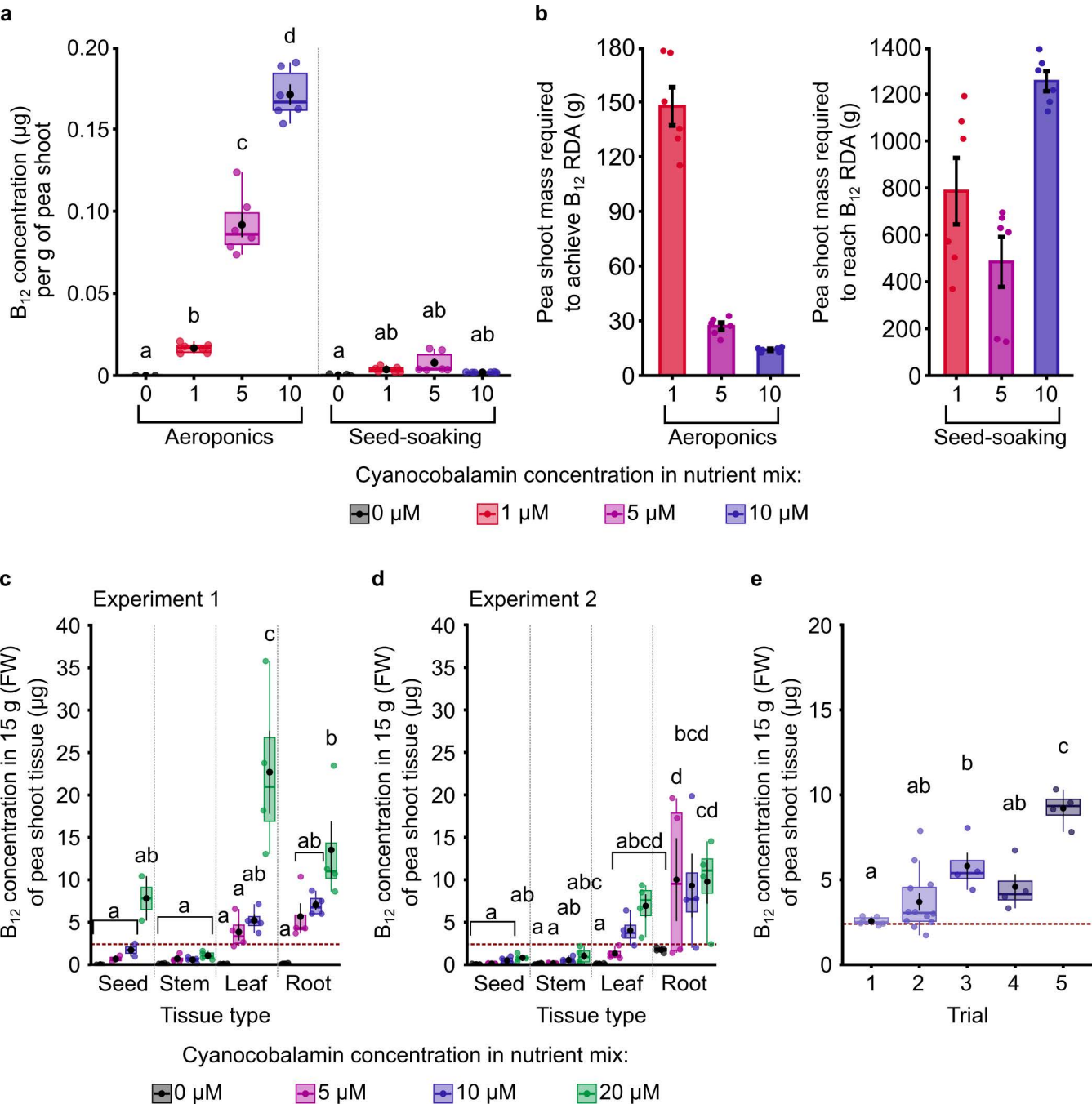
A new and economically-feasible approach was developed for dietary supplementation with Vitamin B12 by a salad crop, through the use of aeroponic horticulture technology.

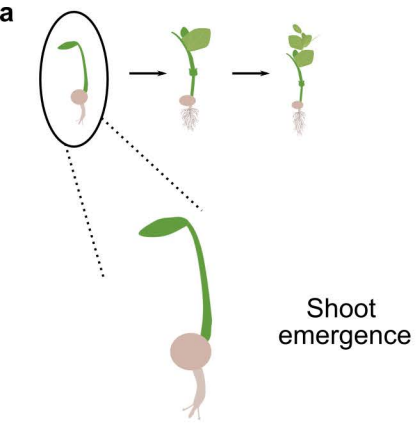
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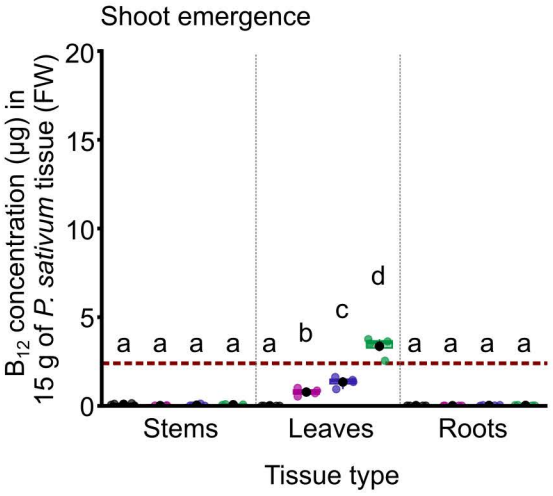
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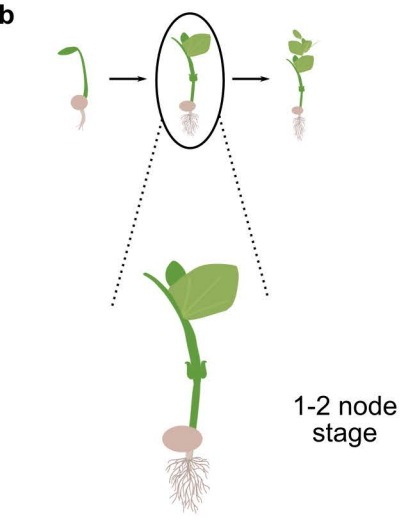

a
Nutrient solution
Cyanocobalamin supplementation
Ultrasonic aeroponic growing system
Harvest

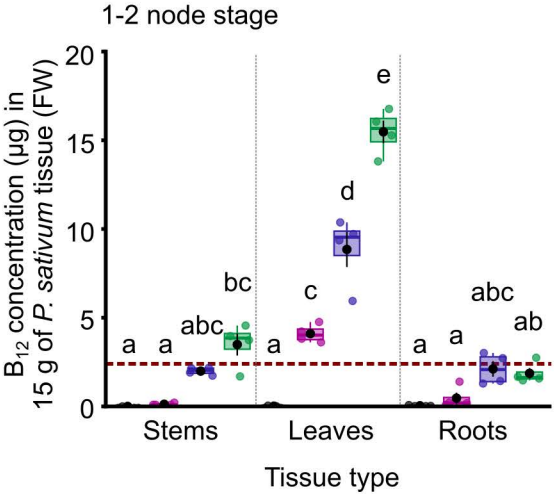

b

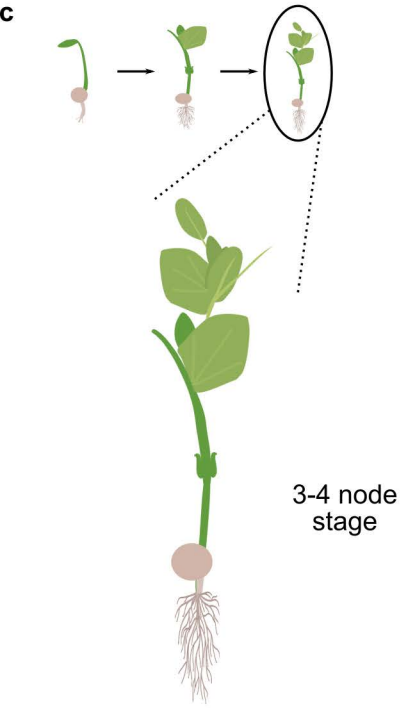

a
b
c
d
B_{12} concentration (µg) per g of pea shoot
Aeroponics
Seed-soaking
Pea shoot mass required to achieve B_{12} RDA (g)
Pea shoot mass required to reach B_{12} RDA (g)
Cyanocobalamin concentration in nutrient mix:
0 µM
1 µM
5 µM
10 µM
Experiment 1
Experiment 2
B_{12} concentration in 15 g (FW) of pea shoot tissue (µg)
Seed
Stem
Leaf
Root
Tissue type
20 µM
Trial

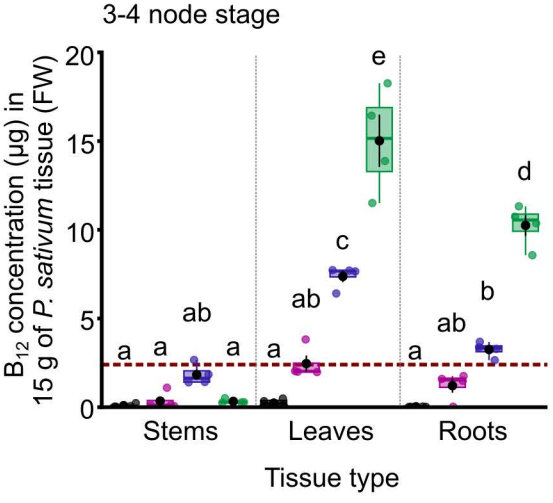

a
Shoot
emergence

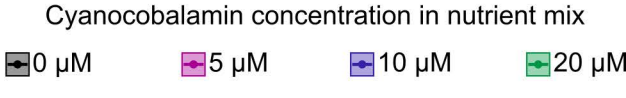

Shoot emergence
B_{12} concentration (µg) in
15 g of *P. sativum* tissue (FW)
20
15
10
5
0
a a a a a b c d a a a a
Stems
Leaves
Roots
Tissue type

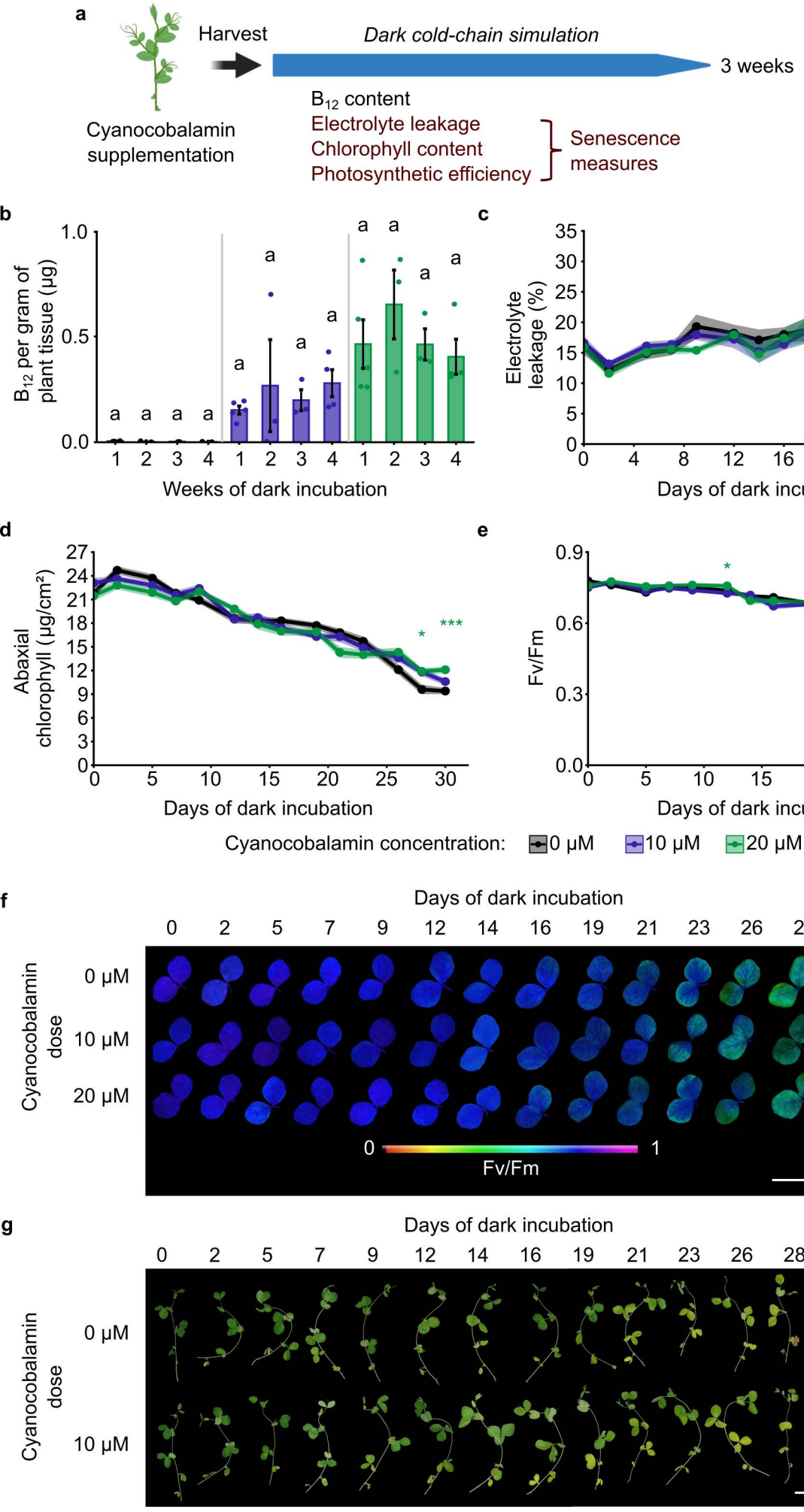

b
1-2 node
stage

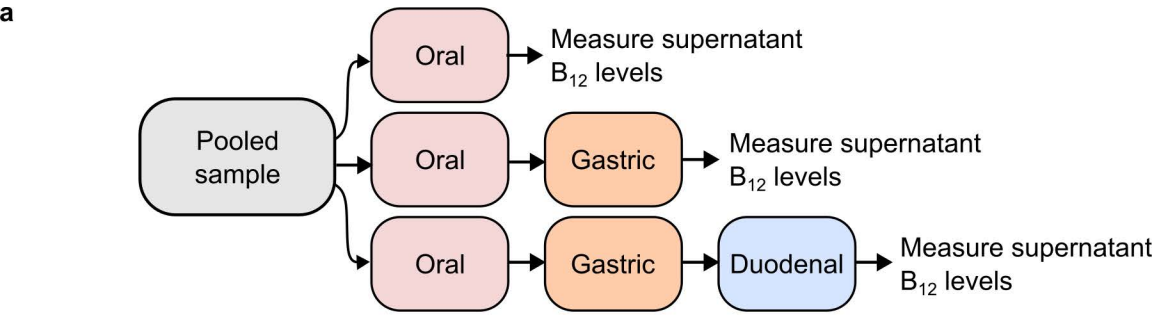

1-2 node stage
B_{12} concentration (µg) in
15 g of *P. sativum* tissue (FW)
20
15
10
5
0
a a abc bc a c d e a a abc ab
Stems
Leaves
Roots
Tissue type

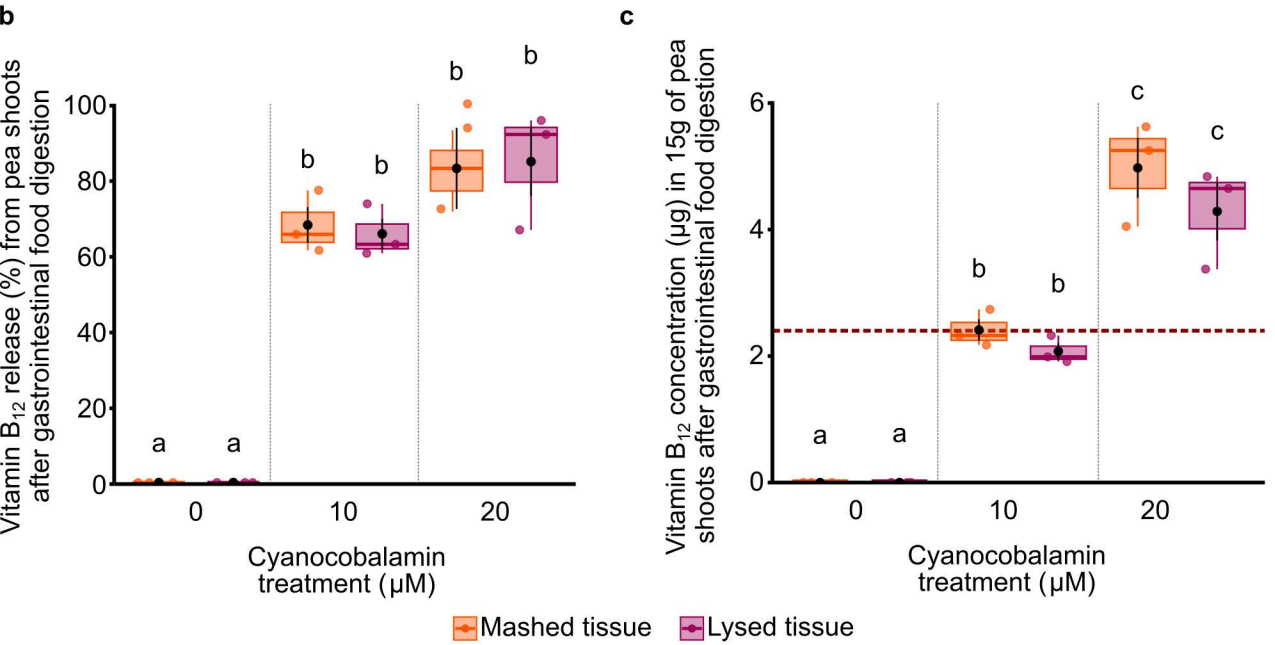

c
3-4 node
stage

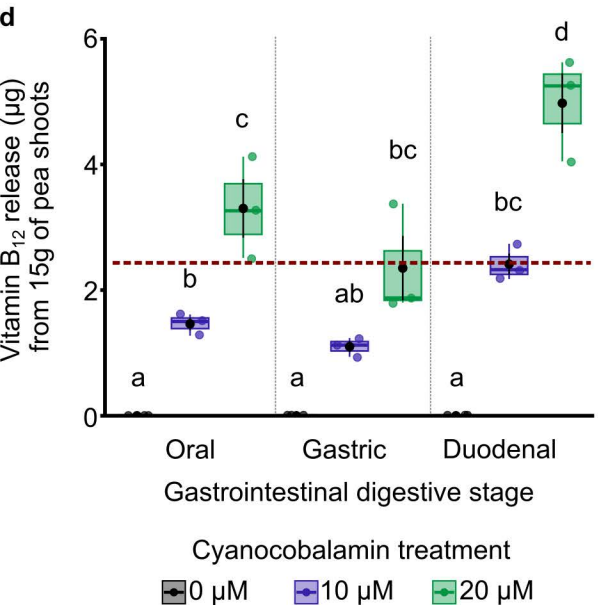

3-4 node stage
B_{12} concentration (µg) in
15 g of *P. sativum* tissue (FW)
20
15
10
5
0
a a ab a a ab c e a ab b d
Stems
Leaves
Roots
Tissue type


Cyanocobalamin concentration in nutrient mix
0 µM
5 µM
10 µM
20 µM


a
Harvest
Dark cold-chain simulation
3 weeks
Cyanocobalamin supplementation
B12 content
Electrolyte leakage
Chlorophyll content
Photosynthetic efficiency
Senescence measures
b
B12 per gram of plant tissue (µg)
Weeks of dark incubation
c
Electrolyte leakage (%)
Days of dark incubation
d
Abaxial chlorophyll (µg/cm²)
Days of dark incubation
e
Fv/Fm
Days of dark incubation
Cyanocobalamin concentration: 0 µM 10 µM 20 µM
f
Days of dark incubation
Cyanocobalamin dose
0 µM
10 µM
20 µM
0
1
Fv/Fm
50 mm
g
Days of dark incubation
Cyanocobalamin dose
0 µM
10 µM
50 mm


a
Pooled sample
Oral
Measure supernatant B_{12} levels
Oral
Gastric
Measure supernatant B_{12} levels
Oral
Gastric
Duodenal
Measure supernatant B_{12} levels


b
Vitamin B_{12} release (%) from pea shoots after gastrointestinal food digestion
100
80
60
40
20
0
a a b b b b
0 10 20
Cyanocobalamin treatment (μM)
c
Vitamin B_{12} concentration (μg) in 15g of pea shoots after gastrointestinal food digestion
6
4
2
0
a a b b c c
0 10 20
Cyanocobalamin treatment (μM)
Mashed tissue
Lysed tissue


d
Vitamin B_{12} release (μg) from 15g of pea shoots
6
4
2
0
a b c a ab bc a bc d
Oral
Gastric
Duodenal
Gastrointestinal digestive stage
Cyanocobalamin treatment
0 μM
10 μM
20 μM