

Release Notes xxxx-xx-xx : : This is an alternative analysis of a simple differential abundance measure between two groups of brains from one recent publication [95] but it points to ambiguity inherent in a lot of data and puts forth a set of realistic considerations for alternatives. The title derives from literature on symbiosis with plants related to a surprising number of the organisms considered. As soon as I can figure out how to download the Bioproject data I hope to look at sequence level analysis. This may be another case of the mixed taxonomy getting in the way of seeing what is really there. A good database on metabolism may be helpful too.

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Brain Microbiome : Make Your Garden Grow? Feed Your Head

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A recent work comparing the post-mortem brain microbiome of those who died with Alzheimer's to controls [95] provides insightful differential measurements of the two states. The authors mostly interpreted results to support a causal role for the associated organisms in disease progression. They found common skin bacteria such as *Staphylococcus epidermidis*, among others, to be more common in AD brains and these occasional pathogen may be one of several contributing organisms yet to be understood. Their results also suggest the diseased brain has different nutritional or metabolic status. The AD brains had lower abundances of organisms prone to overgrowth in benzoate rich conditions (*Pseudomonas*) while the skin bacteria in the AD brains are thought to benefit from low Trp or 5HT (*Cutibacterium*). Other AD associated organisms benefit from higher iron (*Acidovorax*) and methanol (*Methylobacterium*). This work's title derives from the surprising literature linking many of these organisms to plant nutrient uptake which may or may not be relevant in the brain. Observations from pre and peri-natal microbiome work suggest the possibility the AD brains lost beneficial organisms, such as *Acinetobacter junii*, acquired as early as conception. The response of organisms sensitive to tryptophan, iron, and methanol helps validate the microbiome results as being consistent with known properties of the disease state such as microbleed and endogenous methanol increases. The nutrients highlighted by these organisms tend to support earlier interest in uptake of lipophilic neurotransmitter precursor amino acids as well as vitamin K. The bacteria in healthy brain may help nutrient uptake in plants consistent with solubility as a performance limiting issue. Contrarily, histidine is not implicated as an issue while endogenous methanol production may be an important surprise. Methanol may derive from GI bacteria or SAM and methionine. GI bacteria then could still be causal and methionine may be a realtive issue due to loss of other amino acids although I had not explicitly considered it before. Interventions such as dietary absorption aids including surfactants and acids then may be considered to improve nutrient uptake and minimize methanol production. The possibility of symbiotics or commensals suggests caution in antibiotic usage.

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Contents

1. Introduction	3
2. The Extreme Differentially Abundant OTU's	4
3. Microbes, Nutrients, and Disease	7
3.1. Tryptophan related	7
3.2. Tyrosine related	8
3.3. Histidine related	8
3.4. Methanol related	8
3.5. Iron and Vitamin K related	8
3.6. Exposure, Vulnerability, and Uptake into CNS	9
4. Discussion	9
4.1. Informing Interventions	9
4.2. Plant Symbionts in Brain : Coincidence or Nurse Bacteria ?	11
5. Conclusion	11
6. Supplemental Information	11
6.1. Computer Code	11
7. Bibliography	12
References	12
Acknowledgments	22
A. Statement of Conflicts	23
B. About the Authors	23
C. Interpretation Issues- General and Specific	23
D. Symbols, Abbreviations and Colloquialisms	23
E. General caveats and disclaimer	23
F. A baseline nutrient outline	24
G. Citing this as a tech report or white paper	25

1. INTRODUCTION

Despite many interesting discoveries in the lab and clinic, cause and effect in many biological settings remains difficult to determine stifling the design of therapeutic interventions. Alzheimer's Disease is one important unresolved medical issue which may exemplify this limitation. For decades, amyloid beta and tau had both been considered as causal in the sense that removing either one would reduce disease processes and produce significant clinical benefits or even a cure (see citations in any of the AD citations below). That state of affairs is well documented in the works that hint at it unravelling [37] such as a 2002 work suggesting that "tauists" and "baptists" could 'shake hands' and look for other causes [98]. Efforts continued and recently Aducanumab was approved despite trials having been stopped for futility [140] followed by Lecanemab [150] [146]. Other approaches target cholinesterase [47] due to decreased acetylcholine and NMDA receptors [74] to control glutamate and Ca with limited success.

Recently, the infectious disease hypothesis has gained attention [51]. [95] [64]. This is motivated by a variety of observations including the realization that amyloid beta is protective and appears as a CNS specific immune response. A 2022 work concluded AD is an autoimmune disease modulated by Trp metabolites [33]. However, autoimmune disease always invites the "undetected pathogen" concern and hence an interest in infectious etiology. Immune activation and Trp depletion associated with AD were recognized as early as 2000 [156]. Similarly, Parkinson's has been considered as autoimmune with possible undetected infection [30]. Perhaps most interesting is the similarities between various known spirochete infections including *Treponema pallidum* and AD [92] especially as syphilis cases continue to escalate although in younger high-risk fringe groups [4] distinct from the typical AD patients.

Another recent study demonstrated that AD symptoms and reduced adult hippocampal neurogenesis could be caused by fecal microbiome transplant from AD but not control humans [45] tending to support an infectious or transmissible attribute.

Other work has continued on age related cognitive decline in general. Some of this involves microbes in various ways such as the GI microbiome and their metabolites. A mouse model of questionable relationship to real human diseases for example was investigated for behavioral issues related to metabolic and gut microbia properties [90]. In general tryptophan metabolism is now being explored as a mediator from GI microbiome to brain function [41] with particular interest in 5HT [102] Some work appears to just emphasize the role of Trp metabolite and various details [124] without considering the likely context pointing to deficiency.

Significant literature exist on gut bacteria or organisms not within the CNS and their impact on the brain by various means such as metabolite generation or nutrient modifications. Metabolites may also exist in ingested food independent of host microbiome. One interesting work from 2007 identified tryptamine as a cause of neurodegeneration through Trp-tRNA synthetase and the effects may not be overcome with excess Trp [108]. Recent literature on microbially derived tryptamine and indeed tRNA related signalling and particularly Trp-tRNA- related signalling has added details but not resolved issues completely.

A nutritional or dietary component has been considered and various interventions proposed. Not surprisingly given the observations of decreased acetylcholine, dietary choline is a popular choice [149]. In the case of choline deficiency, given that gut organisms are thought modulate choline availability to the host [122] , nutrient and neurotransmitter deficiency could be mediated by infection with the wrong microbes and age dependence due to impairment of GI tract. In this case eradication of the offending metabolic routes may be therapeutic but ultimately the presumed vitamin deficiency still needs to be corrected to try to repair the GI tract if possible.

In any case, microbe exposure is not as age specific as Alzheimer's Disease and an infectious etiology suggests a good intervention will be host directed attempting to restore the successful "youthful" response. There are special events such as conception at which time exposure and response to microbes is unique and difficult to replicate later in life. By finding associations between groups of organisms and clinical trajectory, work on the infectious hypothesis will greatly aid evolving hypotheses that consider the microbial environment such as barrier function and nutrient flows.

The present work builds on my earlier efforts that suggest other neurotransmitters, notably those derived from the "WHY" (tryptophan histidine tyrosine) amino acids, possibly along with vitamin K, are more important although a complete solution will address the age related bottlenecks.

Herein, I describe how common sources like lecithin may also promote absorption of other nutrients making success anecdotes ambiguous. One work suggesting amino acid and tryptophan restriction to counter aging suggests that can impair cognition [19].

The present work seeks to support a nutritional hypothesis, without or without microbial involvement, that cognitive decline and correlates of aging in general are due to effective starvation for various nutrients in compartment specific ways. Part of this was alluded to in a response to covid-19 [84] [86]. Some interest has recently been expressed in age related GI damage suggesting interferon-gamma per se may be part of a problem [103] and a recent analysis of long covid points to serotonin deficiency and nutrient issues [157].

Seemingly unrelated work can be linked to nutritional status. For example, a causal role for lncRNA such as MEG3 in mediating neuron death in AD has been described [6]. You may recall this had previously been implicated in

apoptosis and endothelial cell dysfunction related to high glucose [155] [142] as well as pathogen [113] and tumor control [160] through mTOR. With little direct testing of nutritional interventions, it is interesting that dietary tryptophan was found to improve a glucose issue in fish via lncRNA [53]. Its also worth noting that in the past coding regions had been given the most attention while the more idiosyncratic regulatory issues had been ignored. Control and feedback may be important responses to various stimuli including nutrition but much analysis assumes monotonic static correlations based on protein functions. Another line of attack is recent work with aquaporin 4 pointing to read through [93]. There is some indication linking Trp (and Tyr) or its tRNA to read through rates [12].

The work will interpret the microbiome results from figure 4 in [95] in terms of the literature on the most differentially abundant organisms (those at either end of the curve) which have been listed in Table I. This tends to confirm that their results have some validity as some organisms suggest the observed changes in iron and methanol distribution likely in diseased brain while others relate to differential abundance of nutrients thought to be neurotransmitter precursors such as tryptophan and tyrosine. Further consideration of methanol however adds concern for two possible endogenous sources: GI bacteria and excess methyl donors. Increasing stomach acidity may reduce GI methanol production and that is discussed as a low risk approach to explore although contrary to many notions that encourage PPI usage. Creation from methyl donors motivates a larger interest in controlling nutrients like methionine and supplements like SAM. Further consideration of these organisms in this suggests that some of the differences may be due to loss of symbionts¹ that could have been present since conception and this hypothesis appears to be novel for AD. If they were seeded during conception it may be difficult to re-establish in old age and the use antibiotics would need to be considered carefully.

2. THE EXTREME DIFFERENTIALLY ABUNDANT OTU'S

Table I lists the organisms implied to be associated with health or AD as taken from figure 4 in [95] . they are discussed in an order related to their features. Some have been ignored due to lack of interesting literature although some inferences may be able to be made later by appeal to the 16s sequence later. Host-organism relationships can be quite diverse and many organisms tend to be benign or beneficial to humans but are also found to become pathogenic under conditions that may not be well defined.

Title	
Six OTUs are more abundant in the control group:	
Acinetobacter junii	common in soil animals human water soil, plant growth promoter, from birth?
Comamonas jiangduensis	plant symbiont, genus uses Trp
Cloacibacterium normanense	breast milk aromatic degrading
Pseudomonas putida,	denitrifyin,benzoate overgrowth breast milk
Pseudomonas thermotolerans	may not use benzoate
Diaphorobacter nitroreducens	sludge not benzoate, methanol, or sugars tetracycline favored
Seven OTUs are more abundant in the AD group:	
Cutibacterium acnes	skin resident, overgrowth in 5HT depletion
Staphylococcus epidermidis	skin resident, known pathogen in infant brains
Acidovorax ebreus	Fe oxidation
Acinetobacter tjernbergiae	sludge uses histidine but not others
Acidovorax temperans	Fe oxidation
(Novi)herbaspirillum soli	volcanic burned soil plant enhancer
Methylobacterium goesingense	methanol consumer

TABLE I: Miscellaneous features of OTU's identified in [95] that are more or less common in AD vs control post mortem brain sections after minimal processing. In order of "Extremism" with first entry being the most extreme in each category.

Cutibacterium : *Increased in AD :* Prior to 2016, Cutibacterium acnes was in the genus Propionibacterium with a 2019 proposal for supspecies defendes added to acnes and elongatum which is associated with progressive macular hypomelanosis rather than acne [32]. If it is elongatum, relationship to a pigmentation disorder suggests interaction with tyrosine or tyrosinase. Treatments of hypomelanosis may include benzoyl containing products [66] although transformations remain unknown, C. acnes is thought to be able to synthesize biotin as this has been

¹ The distinction between symbiosis and commensalism may be important but the benefits to the organism are generally assumed and symbiosis will be used by default.

recognized as a skin or hair issue [44] and sometimes comes up in brain health. Presumably then low biotin would favor it and signal an unhealthy environment.

The species is itself quite diverse. Interestingly, it is credited as a pathogen in many infections with a strain-specific antibiotic, thiopeptide cutimycin, which is supposed to kill *S. epidermidis* although the interaction appears to be two-way [15]. An antibiotic would be surprising given that *S. epidermidis* abundance has increased too. This could be a candidate for an AD pathogen but given its ubiquity it would need other changes in the host. I very recent work on fungal infection found *Cutibacterium* expansion in Tph^{-/-} mice which were unable to convert tryptophan into 5HT [121]. Even though this intervention may have raised tryptophan levels, it acted similarly to tryptophan depletion in that the tryptophan metabolite 5HT was missing allowing for proliferation. "Serotonin degeneration" has been implicated in cognitive declines [133] and it may not be clear in which direction cause and effect operate but degeneration in response to limited tryptophan would be quite plausible.

Staphylococcus : *Increased in AD :* Until recently *Staphylococcus epidermidis* was not considered pathogenic as a normal inhabitant of the skin but is now recognized as a common pathogen [105]. If there is a specific pathogen responsible for age associated cognition defects this is the most suspicious of the group. It is the most common infection in pre-term infants and activates microglia and modulates BBB permeability [46]. Like some of the other bacteria here, it has been isolated from planted areas and in particular apple orchards and found to promote the growth of clover [25]. One report suggested it is a symbiont in the female mouse reproductive tract [104]. It was found in higher amounts in infant fecal microbiome when vitamin K deficient [11] which may be expected as "leakage" or diffusion from the skin and reduced coagulation barrier function.

As a coagulase-negative *Staphylococcus*, it can secrete antibacterials such as 6-thioguanine a purine analog inhibiting purine synthesis in susceptible organisms [26]. It would be interesting to check community for overall entropy and susceptibility to see if this is a relevant factor. In particular, determine if *A. junii* is susceptible.

Trp metabolite have some activity against it [100] suggesting its presence may indicate low Trp metabolism due to lack of tryptophan or reduced activity of any surviving brain cells.

Acidovorax : *Increased in AD, 2 species :*

Acidovorax was found in root endosphere of Chinese Chives [137].

As with some other genera, *Acidovorax* interactions with plants are well documented [16]. Beneficial and pathological relationships have been distinguished by genomes with beneficial abilities to sense, transport, and synthesize useful molecules while pathogens have secretion systems and ability to use plant synthesized lipids [130]. Besides acetate, *Acidovorax* can use lactate and citrate as carbon sources [23]. It is notable for oxidation of Fe-II and encrusting mineralization [24]. Although the benefits of iron oxidation have been demonstrated, encrustation depends on iron concentration and perhaps absence of chelators [23]. This may generate a distinctive "green rust" [170] [111]. Arguably precipitation is a better fate for excess iron than some alternatives so even this may be mitigating although not sustainable as minerals accumulate.

(Novi)herbaspirillum : *Increased in AD :* Originally isolated from plants in volcanic ash, and assigned to *Herbaspirillum*, *Noviherbaspirillum soli* is thought to be siderophore producing and plant growth promoting and will assimilate lactate and hydroxybenzoate [22] possibly making it parasitic if it predominantly uses lactate in the sick brain. The distinction between "parasite competing for scarce lactate" and "symbiont benefiting from excess benzoate" is discussed later as there is a lot of ambiguity in all cases without networked rate equations. It is also possible that more lactate is available due to astrocyte production in excess of demand by dysregulated neurons. *H. soli* changed genus circa 2014 from *Herbaspirillum* after proposal in 2013 [71]. A relative, *N. denitrification* HC18, can methylate methyl-As³ but not As³ [167]. Another relative, *Herbaspirillum* sp. WT00C, is an endophytic selenite reducer specific to tea-plants [159]. *Noviherbaspirillum* was one of a few genera to increase in abundance in a soil sample with the addition of pyrogenic organic matter [165].

Species of *Herbaspirillum* considered plant growth promoting bacteria (PGPB) were described as having low siderophore production but P solubilizing ability as endophytes [31].

Methylobacterium : *Increased in AD :* *Methylobacterium* are notable for metabolism of methanol and succinate in beneficial association with plants [115]. Indeed, increased formaldehyde is observed in neurological patients and the elderly [34] suggesting the presence of *Methylobacterium* is indicative of methanol accumulation and perhaps mitigation of formaldehyde damage by oxidizing methanol. It is converted to formaldehyde by a periplasmic dehydrogenase and in the cytoplasm converted to CO₂ or assimilated [139]. Associations with plants may be endophytic or epiphytic and appears to be largely due to methanol released during pectin metabolism [139].

Comamonas : *Increased in healthy :* The *Comamonas* genus was also observed to be less abundant in intestinal microbiome of AD patients [154] suggesting the brain and intestinal abundances may correlate although not mentioned in a similar 2017 analysis [151] or a recent review article [68].

Thinking aloud

check for name changes lol

Comamonas jiangduensis known for producing biosurfactant in agricultural soil [136] They are infrequent human pathogens and some members were originally classified as *Pseudomonas* [123]. *Comamonas acidovorans*, as part of plant rhizobacteria, with tryptophan was observed to promote lettuce seedling root elongation [7] although 10mM tryptophan inhibited its growth.

Comamonas genus is also considered metal tolerant [20]. Potentially then low levels of heavy metals could favor these organisms leading to increases in absolute abundance. Depending on the numbers then, it is possible that some cognitive benefits could result from small amounts of these toxic elements. While perhaps not likely, its important to remember issues like this especially when the obvious interpretations don't lead to useful interventions or "go over like a lead balloon."

Cloacibacterium : *Increased in healthy :* *Cloacibacterium* was found abundant in stem endosphere while Chinese Chives [137]. Isolated from municipal wastewater by 2006 [2], *Cloacibacterium normanense* is electrochemically active [5] [163] Along with *P. putida*, it is a component of breast milk (Guatemalan mothers) and both have aromatic hydrocarbon degrading capabilities [43]. Its route to the milk is controversial however [76].

Cloacibacterium normanense is listed as an endophyte of plants grown in textile waste water [129].

Thinking outloud

check direction and numbers with author, also not right next to *Geobacillis* not *Geobacter* lol.

It was one of the most increased genera in recurrence free patients' BAL fluid prior to resection of early stage non-small cell lung cancer [112] .

Cloacibacterium was detected in an exploration of breast microbiome and metabolites such as TMAO in triple negative breast cancer [153] From their file mmc4, it looks like several p-values are lower than TMAO including lactate, Trp, and Trp derivatives. Exploration of their data may be useful but I Was unable to determine from the supplementary information which samples were "IM." The role of TMAO has been an matter of debate and has featured some of the same issues related to causal role now being explored for brain microbes and metabolites [148]. The genus was also found differentially abundant in certain types of arteritis along with metabolic changes [63].

Thinking outloud

The reference also mentions FISH which may be useful in localization.

Pseudomonas : *Increased in healthy :* *P. putida* is known for flexibility [94] [126] suggesting its presence is not particularly informative without phenotype or mRNA information. It can be denitrifying [56]. However, its potential utility in bioproduction has created a significant amount of literature on it including glutarate sensing and metabolism [168]. While glutarate is a toxic product of amino acid metabolism, it would not be clear if *P. putida* would be more fit in a high or low concentration setting but may be able to remove it. A rough form has been found in biofilms along with *Acinetobacter* species [48]. However, *Pseudomonads* and other soil dwelling organisms are known for degrading benzoate derived from lignin [135] and could also be derived from tyrosine or similar neurotransmitters.

Pseudomonas thermotolerans is notable for possibly not using benzoate [77]. Although the real issue is aromatic amino acid usage and early work did suggest a strain specificity at least with *P. aeruginosa*, "28 of 29 strains grew on tyrosine as a sole source of carbon, whereas only seven grew on phenylalanine as a sole source of carbon" [18].

Diaphorobacter : *Increased in healthy :* *Diaphorobacter nitroreducens* was first isolated from activated sludge in 2002 and found to use polyhydroxybutyrate but no common sugars and notably not used were, " methanol, caprylate, citrate, benzoate, serine, and histidine" [54].

Diaphorobacter along with *Pseudomonas* increased in abundance with addition of tetracycline into a nitrogen reducing reactor with mixed bacteria culture [158].

Acinetobacter : *Species fluctuate :* The *Acinetobacter* distribution appears to be the most confusing with *junii* over abundance in the control group and *Acinetobacter tjernbergiae* in the AD brains. Interestingly some species can live off of tryptophan metabolite kynurenine and both of these species lack a putative Mn transporter [8] (that work [8] also has some details of genetics among the pathogens). *Acinetobacter junii* was the organism most overabundant in the control group. At least in combination with other organisms appears to promote plant growth [107]. Described as "hypertolerant" to metals such as arsenic [88] and lead [61] [132] The *Acinetobacter* in general are described as versatile plant growth promoters with beneficial effects on availability or buffering of nutrients like phosphate and metals [99]. A *junii* is rarely a human pathogen but infections have been documented and more likely in those with prior antibiotic use, invasive procedures, or cancer [164] in preterm infants [73]. Intriguingly, it is much more common in the fecal microbiome of those delivered by vaginal birth than cesarean [110] and exposure of cesarean infants to maternal vaginal fluid improved some aspects of neurodevelopment [171].

Pathogenic species of *Acinetobacter* appear to rely on histidine [75] A *junii* was originally identified in 1985 however by its ability to use L-histidine [58] [14]. It is possible that its presence reflect adequate CNS histidine but refer to Appendix C for a more complete description.

Some works compare the species in good details such as [145] [147]Z although no obvious differences between *junii* and *tjernbergiae* were apparent.

Gene clusters related to pathogenicity of *calcoaceticus-baumannii* have been explored [144]. Substrate selections for *A. tjernbergiae* were listed as, [21]

Using the method of Ka"mpfer et al. (1993), L-arginine, L-histidine and quinate are all used as sole sources of carbon and energy and some strains utilize DL-aspartate and L-leucinamide. cis-Aconitate, pimelate, trans-aconitate, adipate, 4-aminobutyrate, azelate, citrate, glutarate, malonate, oxoisocaproate, suberate, b-alanine, L-aspartate, L-glutamate, L-leucine, L-phenylalanine, L-tryptophan, 4-hydroxybenzoate and phenylacetate are not utilized.

3. MICROBES, NUTRIENTS, AND DISEASE

Organisms or communities may be considered pathogenic, beneficial, or neutral, causal or responders with these attributes defined by the result of the subject's removal from the host. Novel data such as these abundances also needs to be "sanity checked" or validated. There are recurring issues with this kind of data even in this short work and some common caveats are described in Appendix C.

The original work [95] analyzes the data to suggest that a pathogenic organism or community is causing the disease and its removal or attack can have clinical benefit. However, looking at the most differentially abundant OTU's, along with other known or suspected characteristics of the disease state, it is easy to make a case for pre-existing beneficial organisms being replaced by disease related emergent organisms largely reacting to and mitigating a changed environment. The distinction is important because in the former case broad spectrum antibiotics with good CNS penetration may be explored but in the latter case restoration of nutrient flow and targeted eradication or seeding may be more prudent. The easy reconciliation between observed abundance variation and disease properties also helps validate the microbiome results.

Taken together, these differential abundances can suggest that the diseased brain is reduced in the supply of tryptophan or metabolites, benzoate or parents like tyrosine, while having an excess of iron and methanol. Reduced levels of vitamin K and biotin may also be factors.

Most or all of these genera, notably *Methylbacterium*, *Pseudomonas*, *Acinetobacter* and *Herbaspirillum*, have shown some activity as plant growth promoting bacteria (PGPB) [42]. *Comamonas jiangduensis*, more common in health, is known for producing biosurfactant in agricultural soil [136] while *Noviherbaspirillum soli*, more common in AD, is found in volcanic soils and may be an early colonizer presumably suited to lower fertility conditions suggesting in general the disease brain may be nutrient poor.

3.1. Tryptophan related

Excess tryptophan may suppress the skin bacteria, the *C. acnes* and *S. epidermidis*. Reduced tryptophan may be due to decreased intake with age, decreased uptake due to GI or vascular impairment, or decreased signalling to bring peripheral Trp to the brain. Metabolite decreases may be purely due to Trp decrease or decreased functions or host cells and bacteria that would produce metabolites such as 5HT. Similar considerations apply to all nutrients including benzoate and tyrosine with all caveats in Appendix C applying.

Some reference works exist on various aspects of metabolism and taxonomy. One work on tryptophan synthesis and IAA production shows for example the abilities of *A. junii* and *P. putida*, which have IPA pathways, in the context of others [134]. while IAA appears to be deleterious at mg/kg for animal fetus [39] [40] and correlates with cognitive decline in CKD patients [72], to the brain, IPA has been investigated as a therapeutic in phase II clinical trials [116] and its other brain related effects [97][9]. Note that the above "IPA" is indole pyruvic acid NOT indole propionic acid although IPA is used for the latter in at least one work on intestinal production of Trp derivatives suggesting both may be beneficial and that IAA production is known to occur [166]. As early as 1999, indole-3-propionic acid was investigated for effects against AD [27]. It is quite possible that for a beneficial relationship the organism has to adapt its metabolism to the human host versus a typical plant.

As early as 2000, it was demonstrated that acute tryptophan depletion could worsen cognition among AD patients [118] but that does not prove that the dominant cause of the natural disease could be corrected by providing more Trp. A 2003 study of healthy and AD patients demonstrated cognitive impairment by "depleting" trp in a comparison of two amino acid drinks with and without tryptophan [119] suggesting amino acid competition may be significant in the elderly.

A interesting 2010 study in *Drosophila* demonstrated complete recovery from expression of amyloid beta with oral 1,4-naphthoquinon-2-yl-L-tryptophan which was designed based on observations of quinones preventing aggregation [125]. Presumably, vitamin K then could exert similar effects and the compound itself could be broken down to

provide excess tryptophan of unknown significance. It is not know if follow up work occurred. Vitamin K deficiency was also recognized as early as 2001 as a contribut0or AD and cardiovascular disease [3] with modern work continuing to assess the situation [1].

3.2. Tyrosine related

Repeated application of benzalkonium chlorides to the Lascaux Cave created an overgrowth dominated by *Ralstonia* and *Pseudomonas* [10]. This suggests a competitive advantage in aromatic rich settings Benzoate consumption has a putative benefit in dementia [70] and it could act to feed symbiotics or aid absorption of nutrients including tryptophan.

Studies relatiing *P. putida* and *aeruginosa* response to aromtic amino acids as in cystic fibrosid sputum have shown some common and species specific features. [109]. Swimming and surfactant production as a function of amino acid exposuire have been investigated at least for *P. aeruginosa* [55].

Thinking outloud

D-typroinse but only in combination with an antibiotic incredible synegy in kill rates [52]. D-amino acids such as D-serine and D-amino acid oxidase are well known in the brain.

3.3. Histidine related

The only obvious relationship to histidine sugests it is probably adequate or more or less consisitent based on *Aceinetobacter*. See howver Appendix C for general ambiguities.

3.4. Methanol related

The alert to methanol was a surprise but supported by signbificant literature. As early as 2014, exploration of chronic methanol feeding relationship to AD pathology was explored [161] with feeding to monkeys producing tau phosphorylation and amyloid plaques [162] similar to AD .

Interestinly investigation on APOE-4 and ethanol consumption [67] may be consistent with an important role for methanol in AD pathology.

Supplement usage related to SAM may need to be more carefully considered [38].

Thinking outloud

this is a duplucac citation used later

. Balance of the amino acid pool was considered in passing before [84] and perhaps methionine, best known for translation initiation, is over supplied as protein synthesis is hindered by limitations of tryptophan. Some evidence suggests excess methionine may impair memory for example [141]. A link to Parkinson's-like symptoms has already been considered as early as 2010 [65] and investigation into supplement usage may be useful.

Increased methanol production or concentration anyway is known in old age [35] and may point to better nutrient optimization.

Another recent study focused on transmission of AD into healthy young rats with fecal microbiome transplants, found that *Desulfovibrio* were more common in AD microbiome than controls [45]. There appears to be some literature on this genus [131] and may be useful for further understanding of the issue as well as methanol-ethanol interactions.

3.5. Iron and Vitamin K related

The abundance of iron and methanol responsive organisms is also consistent with prior expectations helping to validate their initial results. iron metabolizing organisms would presumably benefit from heme influx due to vascular issues like CAA or microbleeding which is common in AD [49].

Reduced vitamin K may be suspected due to the iron influx which may be due to angiopathy and defective coagulation. Biotin may also be a factor.

Vitamin K in aprticular may not be fully appreciated but it apparently important for CNS sulbatide regulation [138] [117] and the sulfatides thsmelves have important but confusing effects on coagulation [62]. There is a tendency to jump to anticoagulants with the appearance of inappropriate coagulation but it is necessary to isolate damage and more vitamin K may be indicated i[83].

Acidovorax iron oxidation is intriguing as I suspected mineral deposits were part of "old age pathology" but the question remains if the growth benefit is due to extra iron supply from heme suggesting it is just a symptom of a large leakage problem. Initially, I would have suspected calcium minerals and a role for vitamin K.

3.6. Exposure, Vulnerability, and Uptake into CNS

While *C. acnes* and *S. epidermidis* could both be considered possible pathogens, their lifelong residence on the skin appears difficult to reconcile with the age distribution of AD. However, as would be expected with lower quality barriers due to protein quality correlating with old age, their ability to colonize the brain would be approaching that which existed at birth (with vitamin K deficiency lol) or indeed conception.

A junior original topic here, Vaginal microbiome required more study although some indications are existence of organisms over represented in healthy brain.

Intriguingly, it is much more common in the fecal microbiome of those delivered by vaginal birth than cesarean [110] and exposure of cesarean infants to maternal vaginal fluid improved some aspects of neurodevelopment [171].

Possibly the semen carries some of the speculated symbionts [59] [96] making "infection-at-conception" possible. At least one study did find an increased risk of dementia with antibiotic usage [57] and a very recent review provides more context for the issue [152].

It is possible that it is actually a symbiotic organism lost over the lifetime although that too may be triggered by nutritional status.

4. DISCUSSION

This analysis is only an outline of what could become a more complex or rigorous analysis given enough information on organism pathways and metabolic state. However, from this initial manual investigation several interesting observations can be considered.

4.1. Informing Interventions

I outlined a lot of unpublished work in my initial response to covid-19 including the specific problems with low stomach acid in the background of issues with tryptophan, tyrosine, and vitamin K [84]. Some of the issues related to nutrient mix and solubility are explored in [82] [80] [79] [81].

Interestingly this may be produced by GI bacteria making them the pathogens effectively responsible for dementia.

In fact a 2023 work suggested that frailty more than age per se correlated with covid-19 severity as well as cognitive issues [89]. A link between sarcopenia and cognition is suspected in general at least in some populations [69].

While not considered originally, it later turned out that histidine may be a factor in older age and correspondingly in covid-19 [78].

As low blood levels of Trp may not be observed, it turned out that there is some indication of stress transport [78] thought to be similar to the case with biotin where the liver imports less during starvation while brain metabolism is largely preserved [106].

A 2022 literature review tends to support that amino acid fluxes in sarcopenia reflect low tryptophan levels [28],

However, a study showed that the levels of isoleucine, leucine, tryptophan, serotonin, and methionine in the participants with low muscle quality were significantly higher than that in the participants with high muscle quality, which may be attributed to impaired metabolism of amino acids, resulting in reduced uptake of skeletal muscle, and thus increased circulating plasma amino acid levels (15). Inconsistencies in amino acid profiles in patients with sarcopenia will lead to variations in clinical practice and research.

In fact, it is known that Trp consumed with BCAA's may not be well transported to the CNS presumably raising blood levels but not brain levels.

In fruit mashes, fermentation pH can be a large factor in methanol production as well as pectin content [13]. Reduction of acidity from 2.5 to pH 3.5 may almost double methanol content. Interestingly, endogenous production of ethanol from consumed sugars maybe reduced with citric acid consumption [36] while no direct experiments with methanol production have been found in the literature yet. PPI usage has a controversial relationship to dementia in general. One recent study found a significant increase after years of usage [101] and another 2022 study found associations including a bias towards APOE4 carriers [169]. Although some meta-analyses and reviews have concluded the association is not there [17] or only a problem in those using two rather than one type of PPI [143]. In cases of

discordant results, it helps to look at details and meta-analyses may include issues like too low a dose, too little lag time or confounding factors. Results may vary with population too. In general, stomach acid is expected to decrease with age and the use of PPI's is being questioned [91] they may not have much impact. Low stomach acid is just one of many possible conditions that impair nutrient uptake in aging populations. However, it appears to be easy to correct with more dietary acids, such as citric or acetic or phosphoric in diet soft drinks, taken with meals. Chloride sources may include potassium chloride with meals.

Many suspects exist as nutritional contributors from amino acids to vitamin K. Immediate benefits of simple nutrient additions may not be apparent until GI and vascular damage is repaired or uptake aids are added.

Reasons are considered why simplistic interventions have failed and indeed controlled tests of "vitamins" may not reflect real-world anecdotes. In any case, an interpretation based on reaction rather than cause can explain most of the known evidence. If there is a specific organism group driving clinical dementia it still needs to be better defined as there may be risks to symbiotics with broad antibiotic treatments. Further work can be considered to obtain phenotype information such as microbial mRNA analysis and microbiomes of younger brains with known fetal and peri-natal exposure histories. "Infection-at-conception" would be an intriguing concept to explore even if organisms do not persist into old age.

These results are consistent with my earlier work on covid-19 suggesting that nutrient loss due to age related GI damage is a correctable disease driver even if not reflected in blood levels due to vascular damage and stress transport. It is also possible however that prior neuron damage unrelated to nutrient supply caused a decrease in Trp and Tyr mobilization or 5HT synthesis.

Thinking aloud

there is a recurring problem with equating a blood level with a production rate when the sources and sinks or rate equations are not known.

Microbes then could be contributory through many mechanisms. Besides direct infection of the brain and production of toxic metabolites, modulation of nutrient availability could be achieved by direct metabolism and damage to the GI tract. The former is documented for choline and SARS-Cov-2 is likely to cause GI damage possibly related to ACE2 and therefore tryptophan transport. As this likely accumulates with age and reaches a positive feedback stage where lack of nutrients leads to further GI decay, it could explain age distributions. On the other hand, microbes in the brain make a functional measure of the brain environment at least for bacteria. The ecology may be reflected in OTU abundances and phenotype information from mRNA of the more flexible organisms may further help interpretation. Rate equations accounting for sources and sinks of nutrients are probably needed in many cases.

The other indicators are not clean measurements of but do point to the importance of tryptophan and tyrosine availability or metabolism. The ability to metabolize iron in the diseased state is consistent with known vascular issues and helps validate the results and reaffirm vascular weakness and maybe a role for vitamin K and better clotting. Prior work by other groups specialized towards Alzheimer's has been published with encouraging results although well controlled clinical trials have not been performed to the best of my knowledge.

My prior work has concentrated on dogs but required much of the human literature arriving at unpublished outlines such as Appendix F. Previously published dog diets are similar [80] [87] but more specific ones may be out soon. Recent experience has suggested a role for things such as benzoate which may improve solubility and uptake from GI tract or have other benefits [80] and should be a subject of a future work based on experience with dogs. Nutrient context will likely matter as much as amount. A concentrated concern for lipid solubles may be warranted. Phosphoric acid may be a useful digestive aid, at least in humans, with Diet Coke having been used before to aid dissolution of accumulated matter [60]. Note also that "short chain fatty acids" (including butyrate and acetate) often associated with microbiome and health through many specific mechanisms [128], are themselves acids although overall effect on GI tract pH distribution would need to be explored. Interestingly, methanol can also be bioconverted into butyrate [29] which is probably not as beneficial to the organism as methanol oxidation. Some of the regulatory issues have been worked out in controlled settings [50]

Thinking aloud

(and at least one topology with methanol and formate is interesting as functionally the toxic product formate would encourage consumption of the parent)

Additionally, uncontrolled observations during food preparation suggest salmon broth and vinegar both appear to help dissolve components such as hardboiled egg yolk. For humans, ethanol may also be beneficial explaining inconsistent health benefits associated with alcohol consumption as they may only occur when consumed with appropriate food containing otherwise inaccessible nutrients. As ethanol consumption inherently generates methanol while also preventing methanol conversion into toxic formaldehyde [34], the resulting effects may be moderated and could change "sign". The work with dogs continues to focus on combinations similar to deep eutectic solvents [81]. The association of AD with APOE4 may in fact be related to transport of lipid soluble nutrients.

In the case of vascular pathology such as CAA, the best remedy of course is to clear the plaques and restore the normal populations of transporters. This the CAA may itself be due to nutrient deficiencies and eventually correct itself but in the meantime surpluses may achieve similar results by diffusion.

Interpretation in part is difficult due to operating through taxonomy which is a combination of historical observations with some modifications for molecular "closeness." Phenotype and even genotype/plasmids are not known but may be inferred from the 16s sequence and overall ecology likely to host the given abundance sets. Adding mRNA data may reduce ambiguity with expression of nutrient synthesis or acquisition genes or known lifestyles invoked in known environments. Another approach may be to look only at the complete 16s sequence reads and determine if particular fragments segregate to disease or control and if they mean anything about phenotype. This is a bit speculative but there is existing literature to classify organisms based only on 16s patterns.

4.2. Plant Symbionts in Brain : Coincidence or Nurse Bacteria ?

The appearance of plant symbionts in the CNS is intriguing if accurate although it could simply be the result of "literature skew" and coincidence. First, it would be helpful to determine where they are and how they have been missed for so long. Possibly many exist as spores or other condensed or quiescent forms. Consideration of the isolation methods may be worthwhile. If true, it may be purely coincidental but the known structure of the brain involves compartmentalized metabolism with astrocytes already acting as "nurse cells" to provide lactate to neurons for peak energy demands [114]. The existence of prokaryotic nurse cells would not be unreasonable if they are common in the conception environment. Implications for brain evolution could be significant. My earlier work concentrated on GI impairments since they are often ignored features of aging. Vascular issues present another barrier to nutrient exchange with active organs especially the brain. A third barrier could be overcome with substances excreted by microbes as most of the nutrients considered are hydrophobic.

The status of "immune privilege" [120] then may be as much to nurture bacteria as neurons. As a BBB does not exist at conception, that is a unique opportunity to capture symbionts.

5. CONCLUSION

The top-line microbiome patterns of the subject paper [95] can be interpreted as demonstrating a role for symbiotic organisms acquired as early as conception or the peri-natal period combined with measurement of the brain environment properties likely to be important to neuronal functioning. The organisms more abundant in the control brains may thrive in setting rich in amino acid derived neurotransmitter skeletons or may be beneficial for plants and consequentially or coincidentally have similar relationships to brain by aiding nutrient uptake among other functions. They generally are more competitive in environments presumed healthy for the brain and maybe with some toxic metals present. Those more abundant in the AD brain almost exclusively would benefit from presumed deleterious states of depleted 5HT, increased iron (speculating heme derived), and increased methanol from endogenous metabolism. *Staphylococcus epidermidis* is a notable standout however as a potential pathogen able to cause brain damage. Further work should explore larger patterns of possible pathogen involvement in clinical progression. While likely coincidental, the growth of axons seeking connections and roots seeking nutrients may have some similarities that facilitate symbiotic associations.

A lot of unrelated results can be unified into a nutritional framework with tryptophan being one of the preeminent recurring components. Earlier predictions emphasizing Trp, Tyr, and vitamin K for covid-19 have not been significantly tested but evolving evidence explored in the light of cause and effect rather than a specific coincidence supports their utility in old age conditions. Further work on microbial patterns including metabolic phenotype may be helpful. Nutritional experiments probably need to be more comprehensive including several nutrients, excluding others, and including solubility enhancements perhaps similar to those employed by the organisms overabundant in the healthy brains. Microbiomes from a wider variety of brains including much younger may help determine if any organisms are hosted during early life. A lot of metabolic information exists in the literature in scattered forms perhaps AI would be a good way to investigate nutrient patterns. And its important to continue to question assumptions just in case lead can make your garden grow and feed your head.

6. SUPPLEMENTAL INFORMATION

6.1. Computer Code

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Acknowledgments

1. Nikki Schultek for brining this work [127] as well as the infectious hypothesis to my attention.
2. Pubmed eutils facilities and the basic research it provides.
3. Free software including Linux, R, LaTeX etc.
4. Thanks everyone who contributed incidental support.
5. I have to credit my own software such as bash scripts and of course TooBib[85] for facilitating citation discovery.

Appendix A: Statement of Conflicts

No specific funding was used in this effort and there are no financial relationships with others that could create a conflict of interest.

Appendix B: About the Authors

This work was performed at a dog rescue run by Barbara Cade and housed in rural Georgia. The author of this report ,Mike Marchywka, has a background in electrical engineering and has done extensive research using free online literature sources. I hope to find additional people interested in critically examining the results.

Appendix C: Interpretation Issues- General and Specific

However, increased abundance simply indicates comparative fitness increase. Assuming histidine is limiting, that may be due to increased supply due to host signalling or a virulent organism releasing it or failure of senescent neurons to compete. All fitness arguments and static levels need to be carefully considered with rate equations that include feedback for source control.

While trying to generate hypotheses about cause and effect in order to ultimately design a useful intervention, its important to remember some caveats specific to these data and ambient nutrient levels. The features and citations picked out for this work may be influenced by literature skew towards trendy topics and selection bias. The data are all relative abundances so no real inferences about absolute amounts can be made and all the processings steps employed by the original authors have not been explored. Much of the analysis will now depend on reasoning such as "nutrient X favors organism Y" but the available amount of "X" could itself be due to many factors. Ideally a rate equation should be written to account for all sources and sinks of "X" but even in that case both are likely to be controlled by feedback mechanisms. Tryptophan along with biotin have been shown to be exported in times expected to beneeded by the brain but transport into the CNS may be impeded.

The relationship between microbe relative abundance, nutrient status, and brain health could be quite complicated even if fitness could be easily related to nutrient concentration. The limiting nutrient could exist in amounts determined by various feedback signals from functioning brain or be limited by supply and vascular function. High concentration could even reflect low uptake by neurons in some diseased states. A rate equation with the right control terms could show the complexity of inferring cause and effect.

Appendix D: Symbols, Abbreviations and Colloquialisms

TERM definition and meaning

Appendix E: General caveats and disclaimer

This document was created in the hope it will be interesting to someone including me by providing information about some topic that may include personal experience or a literature review or description of a speculative theory or idea. There is no assurance that the content of this work will be useful for any particular purpose.

All statements in this document were true to the best of my knowledge at the time they were made and every attempt is made to assure they are not misleading or confusing. However, information provided by others and observations that can be manipulated by unknown causes ("gaslighting") may be misleading. Any use of this information should be preceded by validation including replication where feasible. Errors may enter into the final work at every step from conception and research to final editing.

Documents labelled "NOTES" or "not public" contain substantial informal or speculative content that may be terse and poorly edited or even sarcastic or profane. Documents labelled as "public" have generally been edited to be more coherent but probably have not been reviewed or proof read.

Generally non-public documents are labelled as such to avoid confusion and embarrassment and should be read with that understanding.

Appendix F: A basline nutrient outline

Title			
Ingredient	Amount	Relevance	Refs/Notes
Lysine HCl	2600mg/day		rotate K/R
Arginine HCl	1300mg every other		rotate K/R
Threonine	1300mg/day		
Leucine	650mg/day		rotate BCAA
Valine	800mg/day		rotate BCAA
Isoleucine	800mg/ every few days		rotate BCAA
Methionine	250mg/ day		
Histidine	340mg/ day		
Tryptophan	500mg/ day		
Phenylalanine	500mg/ day		not F+Y no BH4
Tyrosine	500mg/ day		not F+Y no BH4
Glutamine	0mg/ day		does not seem to help
Taurine	1800mg/ day		
Lecithin	1800mg/ day		choline and emulsifire
citric acid			
acetic acid			
KCl			
benzoate			mix likely matters
sorbitol			absorption aid
vitamin K1	10mg/day		rotate lipid solubles
vitamin K2/MK4/MK7	10mg/day		mix probably matters
zinc	every other day		
magnesium	400mg/day		see text, citrate+citrate or other
copper	5mg every other day		
Iodine	1mg every few days		rotate SMVT

TABLE II:

Title			
Ingredient	Amount	Relevance	Refs/Notes
B-1	100mg/day		
B-2	400mg/day		reactive, see text
B-3	100mg/day		niacin may be better, see text
B-6	100mg/day		
B-12	1mg/ every few days		out compete cancer etc
folate	1mg/ every few days		out compete cancer etc
biotin	10mg/day		rotate SMVT
calcium pantothenate	500mg/day		rotate SMVT, beta-alanine ok too
lipoic acid	200mg/ every few days		rotate SMVT
vitamin C	sporadic		avoid benzoate
vitamin A	sporadic		rotate lipid solubles
vitamin D	sporadic		rotate lipid solubles
turkey			
chicken thigh			
ground beef 7-20 pct fat			
shrimp			
tuna			
salmon			
Eggland eggs			
spinach			
carrot			
garlic			
EV olive oil			

TABLE III:

Appendix G: Citing this as a tech report or white paper

Note: This is mostly manually entered and not assured to be error free.
This is tech report MJM-2023-008.

Version	Date	Comments
0.01	2023-09-17	Create from empty.tex template
-	October 20, 2023	version 0.00 MJM-2023-008
1.0	20xx-xx-xx	First revision for distribution

Released versions,
build script needs to include empty releases.tex

Version	Date	URL

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run-date = {October 20, 2023} ,
title = {Brain Microbiome : Make Your Garden Grow? Feed Your Head } ,
author = {Mike J Marchywka } ,
type = {techreport} ,
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date = {October 20, 2023} ,
startdate = {2023-09-17} ,
day = {20} ,
month = {10} ,
year = {2023} ,
author1email = {marchywka@hotmail.com} ,
contact = {marchywka@hotmail.com} ,
author1id = {orcid.org/0000-0001-9237-455X} ,
pages = { 26}
}
```

Supporting files. Note that some dates,sizes, and md5's will change as this is rebuilt.

This really needs to include the data analysis code but right now it is auto generated picking up things from prior build in many cases

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