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Tree Assimilation of Nitrogen

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Adequate availability of nitrogen in soils is but one problem facing trees. Moving nitrogen inside a tree presents a number of unique constraints and solutions. Transporting some materials across root cell membranes can be accomplished by simple diffusion where materials move from high concentrations to low concentrations. Transport can also be associated with moving electrostatically charged ions from places with like-charges to places of opposite-charges. Active transport systems are used by root cells for nitrogen uptake.

Active transport mechanisms require energy for maintenance and for moving individual items. These transport mechanisms function both at cell membranes and at internal tonoplasts (vacuole membrane). Carriers on membranes are used to move materials, while cells attempt to maintain a near neutral internal electric charge balance. Active transport is required for uptake of anions ($NO3^-$, Cl^-, $H2PO4^-$, $SO4^{2-}$); excretion of selected cations (Na^+, Ca^{++}, Mg^{++}); and, not required for potassium ions (K^+) which serve for universal charge balancing.

Nitrate Forms

Ecologically, most nitrogen compounds are quickly converted to nitrates under aerobic conditions in soil. Ammonium is a small molecule, easily available on exchange sites in soil, and is already in a reduced state. Unfortunately, ammonium can quickly initiate toxicity problems. Urea can be taken up as applied to a limited extent, but is quickly (3-5 days) converted to ammonium in soil. Urea should not be applied directly to soil or plant surfaces, especially turf, or high pH soils. Various types of nitriform products bind nitrogen within various length carbon chains which are broken apart biologically releasing nitrogen.

Nitrate ($NO3^-$), and to a lesser degree ammonium, uptake into a tree from soil is an energy dependent process. Nitrate is taken into a tree against concentration, hydrostatic pressure, and electrostatic charge gradients. This uptake process is not simple diffusion, but an active process requiring energy to transport nitrate across cell membranes, as well as energy to produce and maintain a carrier system.

Carried

The presence of nitrate in soil stimulates carrier activity (and its own uptake by a tree.) Nitrate uptake also stimulates production of nitrate reduction machinery inside root cells. Nitrate presence in the root area is a signal to a tree to expend energy for transport and processing of nitrate. Tree roots without adequate energy and carbon chain supplies will be stressed by nitrate presence in soil. Actual nitrate uptake is usually much less (4-5X) than full nitrate uptake capacity of tree roots.

Nitrate can not move into tree root cells passively with water as do many of the other essential elements. Nitrate, because of its mass, size, and charge must be actively transported into tree root cells using one of three specialized carrier systems:

1) a low capacity carrier always available and present in the absence of nitrate;
2) a inducable carrier generated under low nitrate concentrations. (Note this carrier is not generated and maintained unless there is nitrate present in the rhizosphere.); and,
3) a carrier which functions under high nitrate concentrations.

Easing Transport

Nitrate is transported using carriers which breach cell membranes. Figure 1 shows two primary types of carriers used by tree cells to move nitrate from one side of a membrane to the other (either apoplast / symplast interface or symplast / symplast interface). Symporters move two items in the same direction usually maintaining a balanced charge across the membrane. Antiporters move two items in opposite directions usually maintaining a mass and / or charge balance.

Nitrate can be moved into a tree by a symporter carrier, where a molecule of nitrate (NO3-) is transported into a cell simultaneously with a proton (H+) to maintain the balance of electrostatic charges. This leaves an OH- outside in the soil. Nitrate (NO3- mw = 62 mass units) is primarily transported by antiports which move nitrates into a cell and an ion of like charge (and similar size) outward. This co-transported ion of similar charge and size is a carbonate anion (HCO3- mw = 61 mass units) from organic acid origins. The presence of HCO3- inside root cells stimulates nitrate uptake and is essential for operation of the primary nitrate carrier.

Ammonium is positively charged and small enough to enter tree root cells with water. Because of nitrogen demands, ammonium ions can also be actively transported into tree root cells using two types of carriers, one transports ammonium under low concentrations and one function at high ammonium concentrations.

Nitrate Reduction

Once inside root cells, nitrate is reduced by a nitrate reductase (NRe) enzyme, which is the first step in reducing nitrates into a usable form. Figure 2. This process is accelerated and maintained by light, CHO, cytokinin, high CO2 concentrations, and anaerobic conditions. This process is inhibited by darkness, transport amino acid (i.e. glutamine) buildup, oxygen, magnesium ions, and low CO2.

NRe is a monstrous, enzymatic catalyst which requires molybdenum (Mo) and iron (Fe) to function. This enzyme is the only major use for Mo in a tree and its requirement can be circumvented (if required) by adding only ammonium-based nitrogen. NRe is energy-expensive to construct and maintain with a half-life of only a few hours. NRe activity is initiated within 40 minutes of nitrate presence in soil and reaches a maximum after three hours. It is estimated up to 25% of tree energy from photosynthesis is used in nitrate assimilation. The only purpose of NRe is to make the initial reduction step on nitrate.

To accomplish the first step in nitrogen reduction, NRe facilitates transfer of two electrons (energy from carbon respiration) to nitrate (NO3-), which yields nitrite (NO2-). This process occurs in root cell cytosol. When excess nitrate is available, nitrate is moved beyond the initial cell of uptake and reduced in xylem parenchyma. In some tree species, a small portion of nitrate (usually the remains of excessive loads) are shipped to leaves for reduction. NRe is not a limiting factor for tree nitrogen utilization, but availability of nitrate to roots is limiting.

Nitrite Reduction

Nitrite (NO_2^-) is moved quickly to plastids (cell organelles) to minimize toxicity. The next three steps in nitrogen reduction is facilitated by nitrite reductase (NitRe), an enzymatic catalyst which requires iron (Fe) and sulfur (S). NitRe facilitates the reduction of nitrite by transferring six electrons (3 electron transfer molecules -- ETMs) which yields ammonium (NH_4^+).

Ammonium can easily become toxic in a cell and is not readily stored. Ammonium is not normally transported in xylem unless large amounts are present and can not be processed in roots. Ammonium is quickly moved into an organic framework (carbon skeleton) with the result called an amino acid. Figure 3. Amino acids can be used as building blocks for other compounds, for storage, or for transport out of a cell and throughout a tree. Cellular assimilation of ammonium ions requires a strong and continuous carbon chain source transported from the photosynthetic process and from local storage materials. Figure 4.

Locking-Up Ammonia

Because of ammonium toxicity, there are two primary pathways (and one secondary pathway) for utilizing this reduced nitrogen compound. Figure 5. The first primary system for incorporation of ammonium into an amino acid is called the glutamate cycle (requiring magnesium (Mg) and zinc (Zn)). Ammonium is added to glutamate (a 5C1N amino acid) and energized (with 1 ATP) to produce glutamine (a 5C2N amide), which is a transport and storage form of nitrogen. Figure 6.

Glutamine (5C2N) and oxoglutarate (5C) are then combined to produce two glutamates (two 5C1N). Figure 7. One of the two glutamates generated is used to start the ammonium assimilation process again, while the other is shipped away for tasks like protein synthesis. The net result is one ammonium successfully incorporated into an amino acid which can be stored or used to transport nitrogen throughout a tree.

Glutamate (5C1N) is the feedstock leading to the other 19 amino acids (actually 18 amino acids and 2 amides) a tree's life is built around. There are over 200 amino acids in trees, although only 20 are structural forms used for enzymes and proteins. Glutamate is the starting material for amino acids, proteins, nucleic acids, nucleotides, coenzymes, and porphyrin rings (chlorophyll and phytochrome). Both glutamate (5C1N) and glutamine (5C2N) can be safely stored for later use or transported to the rest of a tree.

Alternative Assimilation

The second primary system for incorporation of ammonium into an amino acid is a direct carbon chain addition when ammonium ion concentrations are at high levels approaching toxicity (ammonium overdose). Ammonium is added to oxoglutarate (a five carbon organic acid from photosynthesis -- 5C) to generate a glutamate (5C1N). This pathway quickly moves toxic ammonium into storable and transportable glutamate (5C1N), but is energy-expensive and only functions at relatively high levels of ammonium. Figure 8. Figure 9 shows a further step in shifting amino nitrogen to another storage and transport form called asparagine (4C2N).

Third Assimilation

A third ammonium incorporation pathway (of secondary importance) is the aspartate / asparagine process. This pathway is usually used for transferring amino-nitrogens, not assimilating ammonium ion. Ammonium is added to the amino acid aspartate (4C1N) and generates asparagine (4C2N), a amide transport and storage form of nitrogen. Asparagine is used for nighttime nitrogen storage. In daylight, and with strong protein synthesis in the tree, aspartate is continually moved to asparagine. Figure 10.

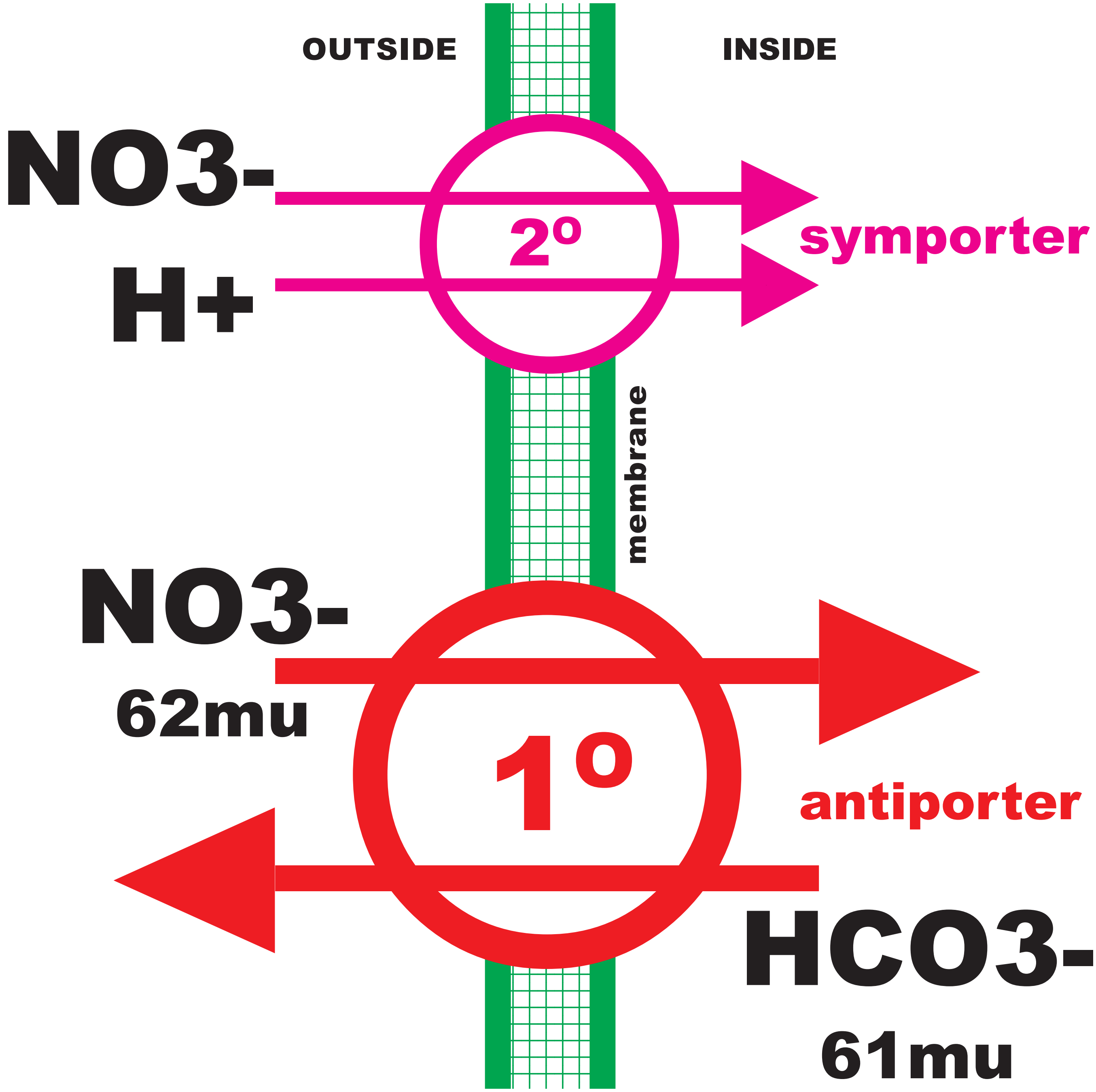

Figure 1: Secondary nitrate (NO3-) symport carrier transports nitrate and proton (H+) simultaneously into cell, maintaining electrostatic charge balance. Primary nitrate (NO3- & 62 mass units) antiport carrier transports nitrate inward and carbonate (HCO3- and 61 mass units from organic acids) outward, maintaining a mass and charge balance.

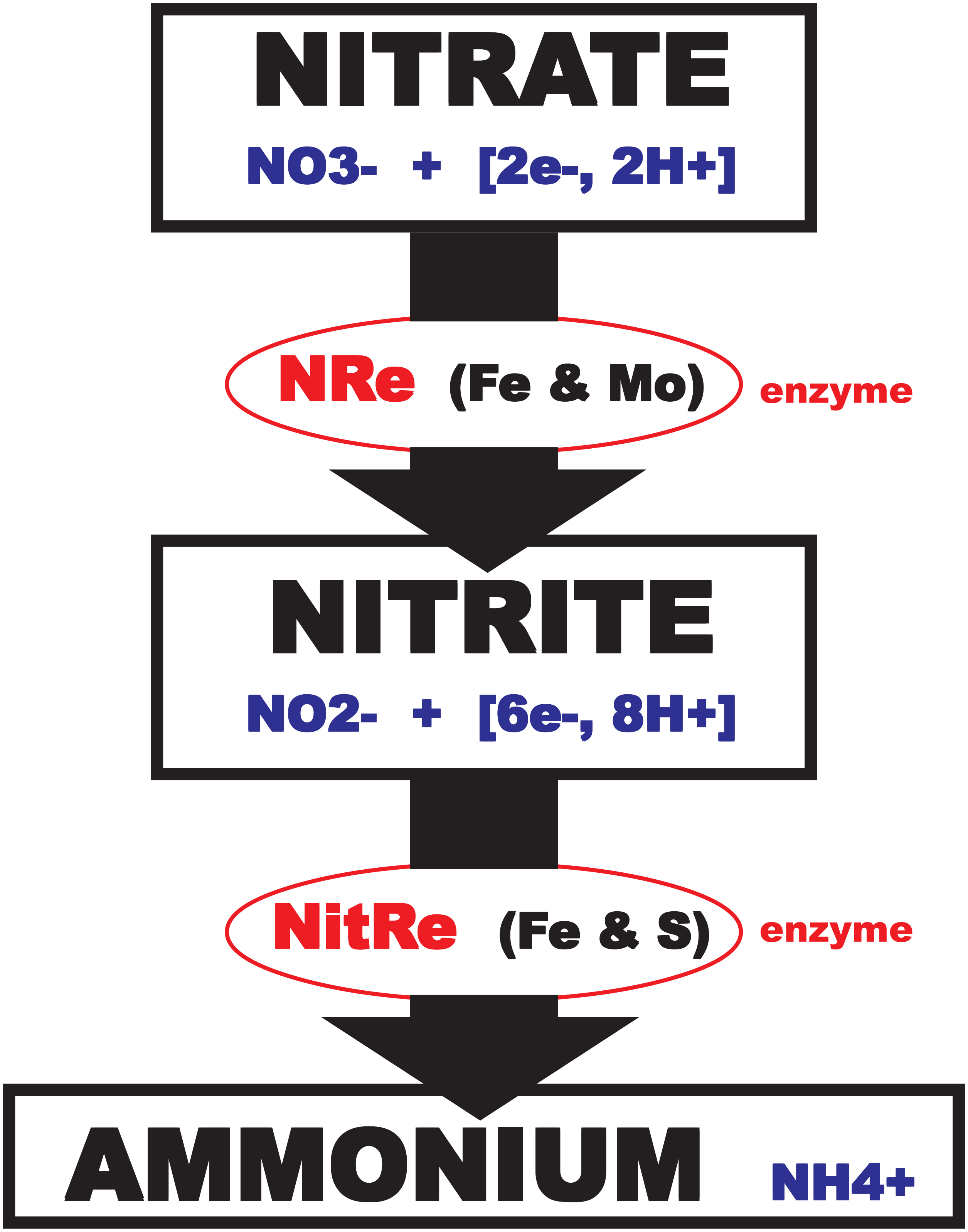

Figure 2: Pathway inside tree cells moving nitrate taken from soil to ammonium ions sequestered in cellular pool.

STRUCTURAL AMINO ACIDS

idealized formula	name
2C1N	glycine
3C1N	serine
3C1N	alanine
3C1N	cysteine (S)
4C1N	aspartate
4C1N	threonine
4C2N	asparagine (A)
5C1N	glutamate
5C1N	valine
5C1N	methionine (S)
5C1N	proline
5C2N	glutamine (A)
6C1N	leucine
6C1N	isoleucine
6C2N	lysine
6C3N	histidine
6C4N	arginine
9C1N	tyrosine
9C1N	phenylalanine
11C2N	tryptophan

Figure 3: Basic amino acids used in trees for enzyme and protein structures (considered structural amino acids).
"C" = carbon; "N" = nitrogen; (S) = sulphur containing; (A) = amide.

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NH4+ INSERTION POINTS

normal ammonium processing

1N(NH4+) + 5C1N = 5C2N

(glutamate) (glutamine)

high ammonium processing

1N(NH4+) + 5C = 5C1N

(oxoglutarate) (glutamate)

occasional ammonium processing

1N(NH4+) + 4C1N = 4C2N

(aspartate) (asparagine)

Figure 4: Three primary ammonium ion insertion points onto carbon chains generating amino acids.

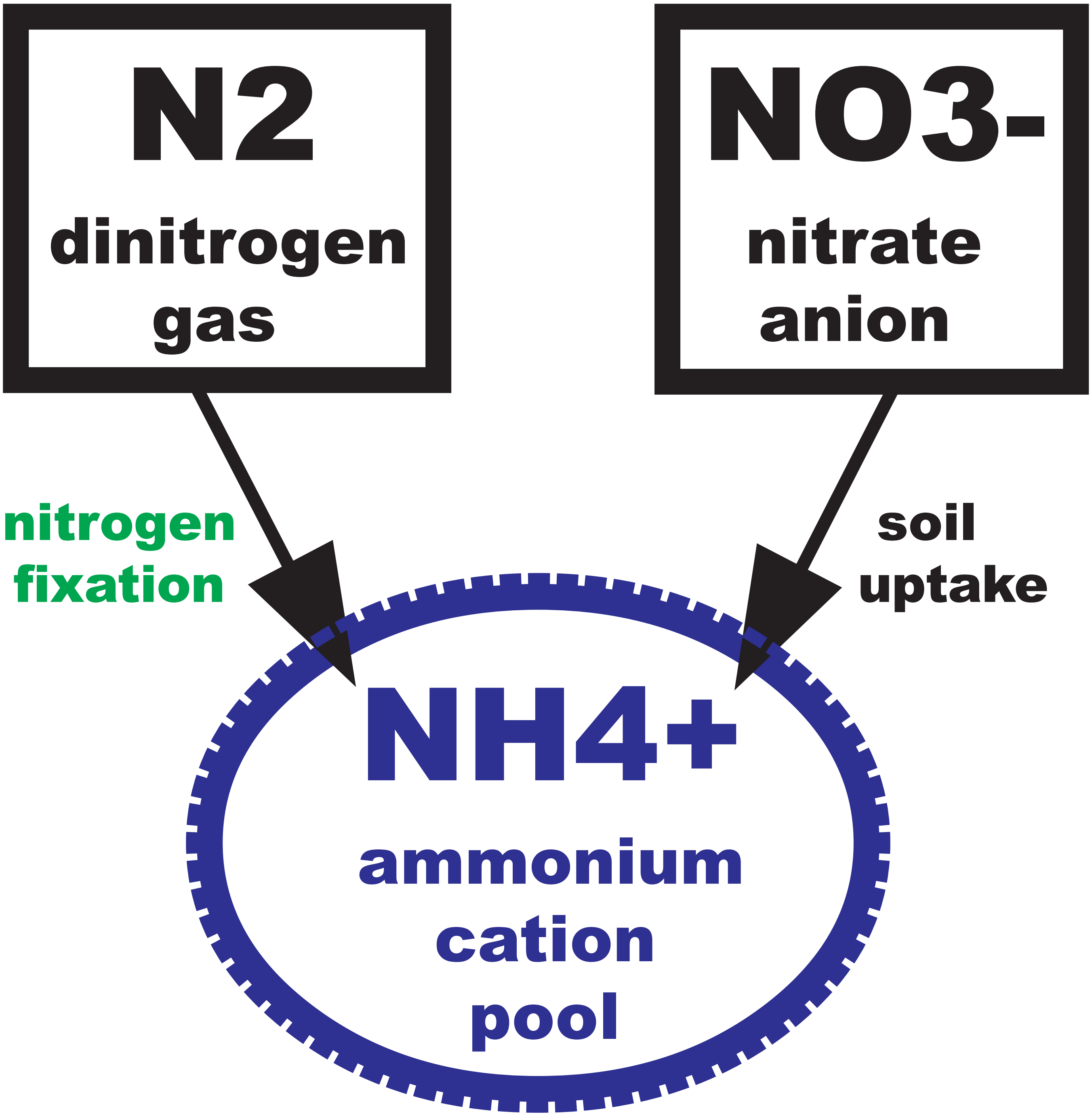

Figure 5: Primary external nitrogen sources supporting the ammonium cation pool inside tree root cells.

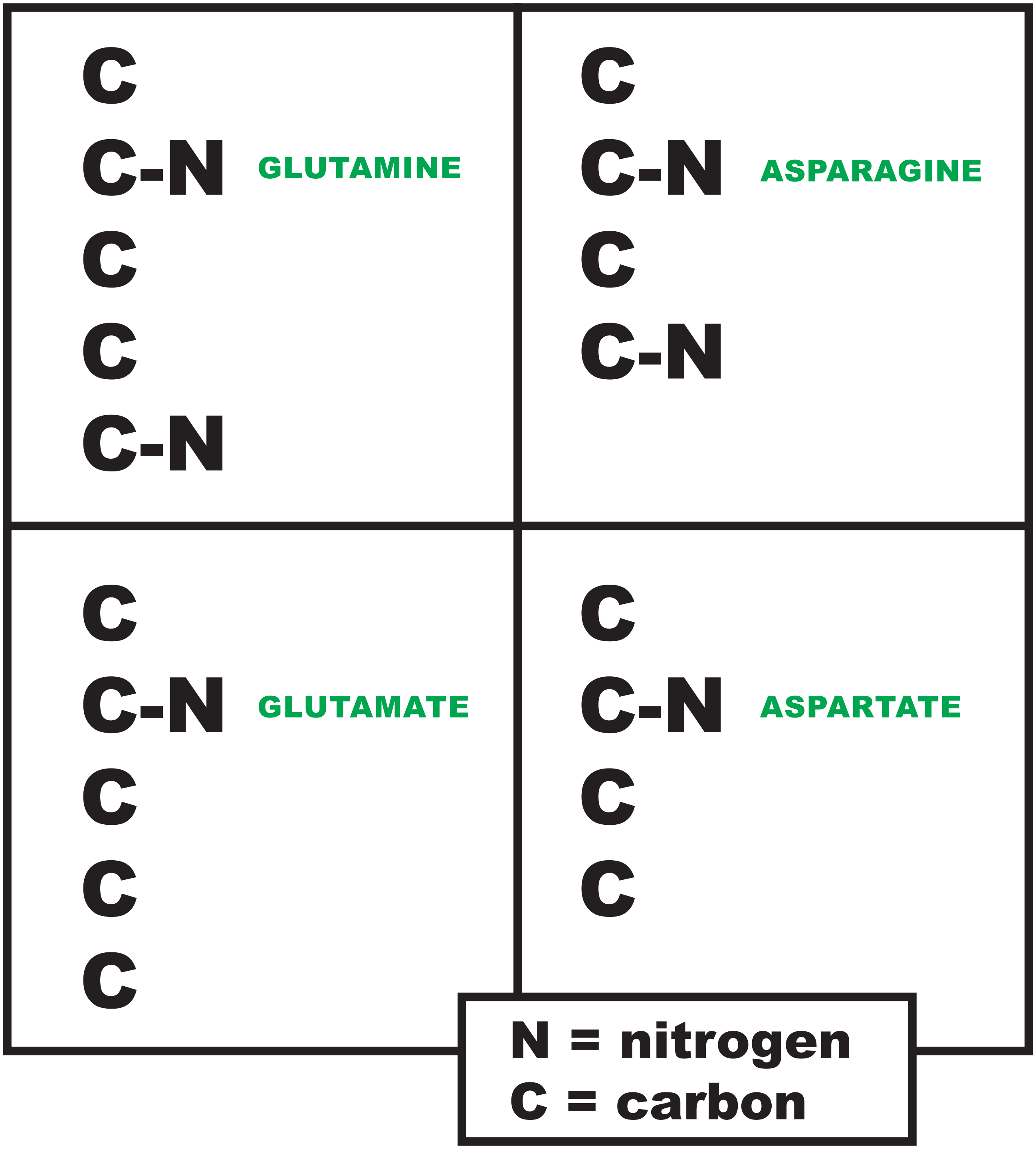

Figure 6: Example idealized structure of nitrogen transport and storage amino acids showing the location and number of nitrogen attachments.
(Structural amino acids can carry 1, 2, 3, or 4 nitrogens.)

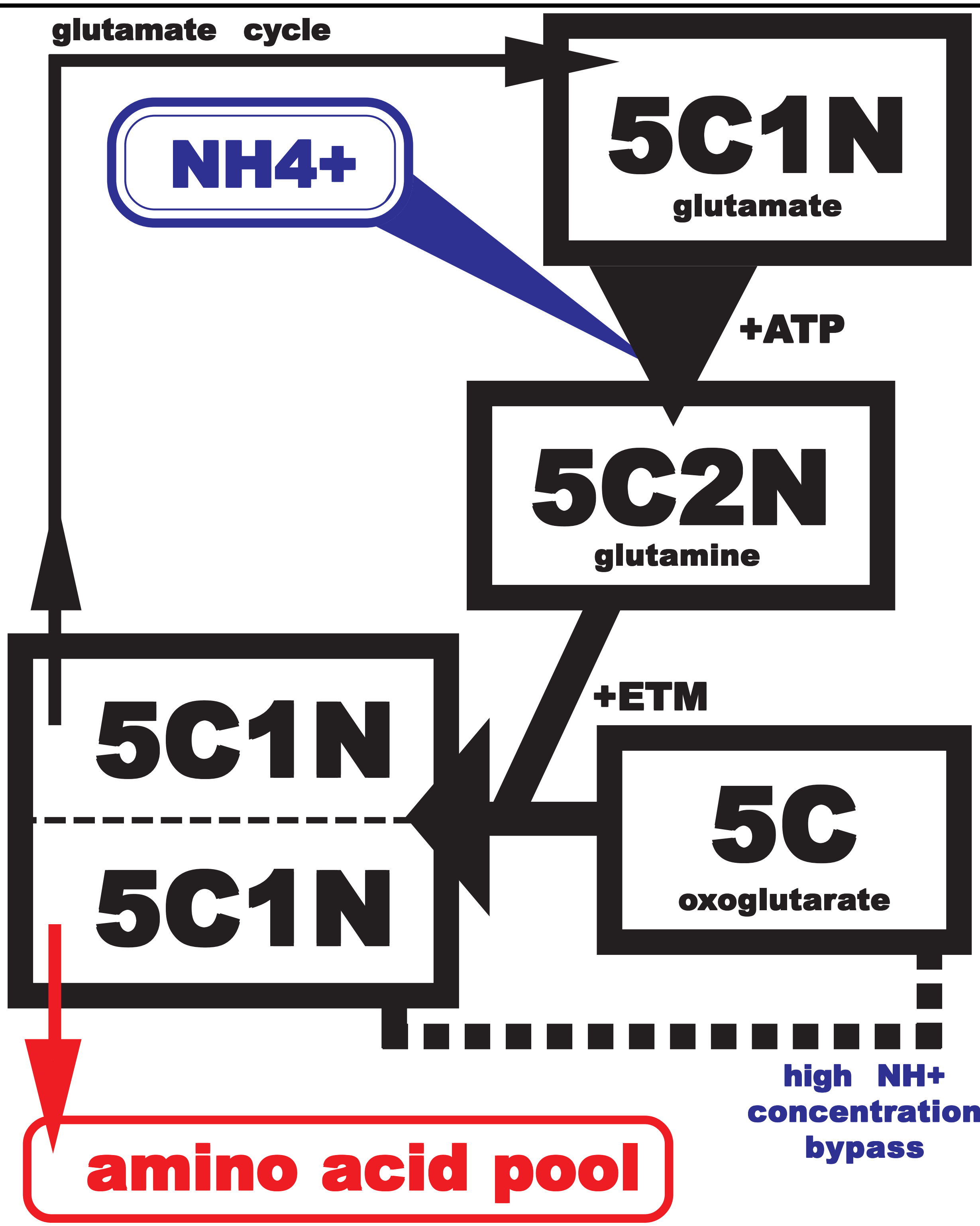

Figure 7: Pathway inside tree cells for accumulated ammonium ions (NH4+) being consolidated into a carbon framework generating two amino acids. (i.e. glutamate cycle)

High NH4+ Concentration Bypass

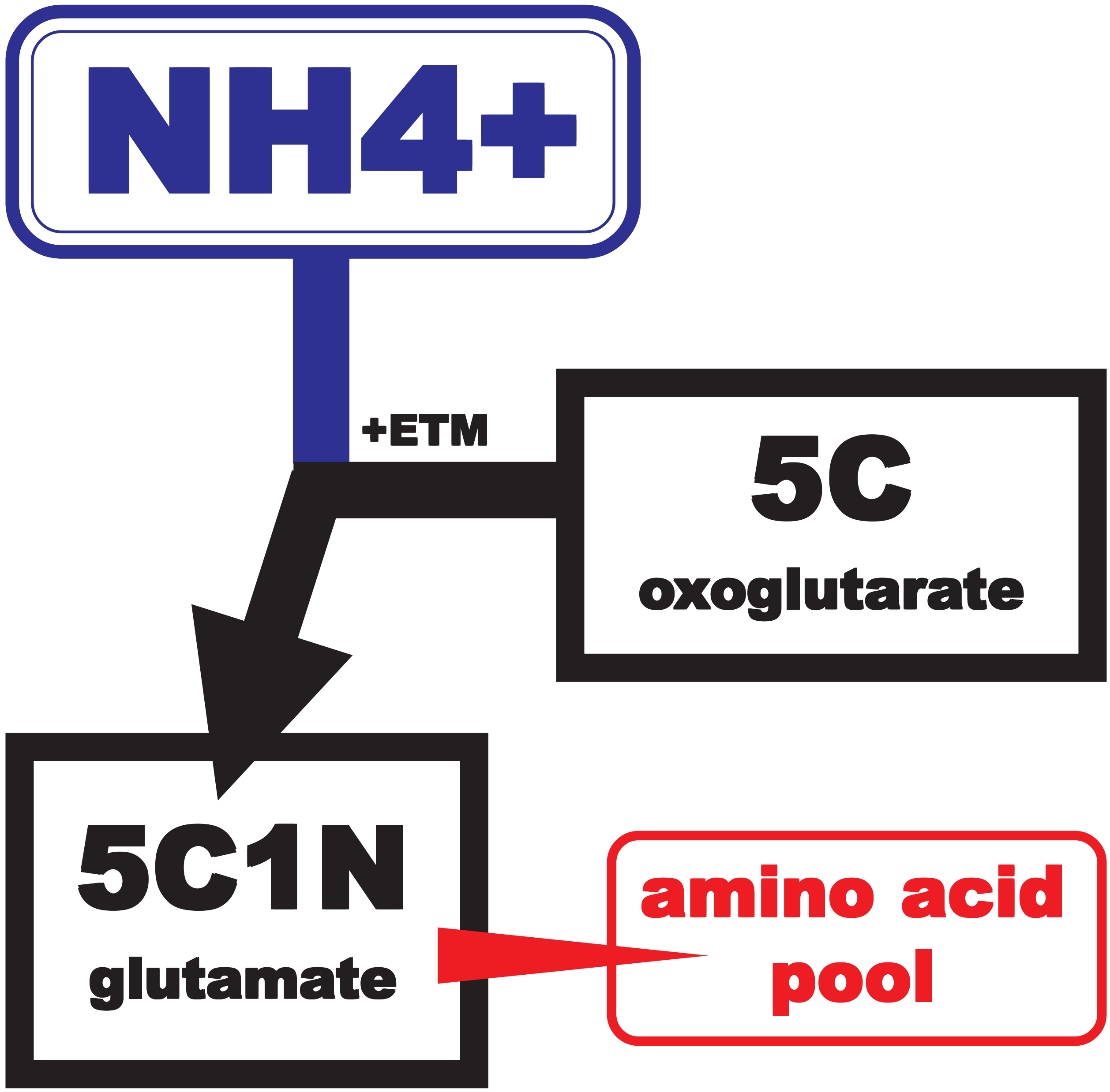

Figure 8: Pathway inside tree cells for high concentrations or excess ammonium ion (NH4+) accumulation being consolidated into a carbon framework generating one amino acid. (i.e. high NH4+ concentration bypass)

LARGE NH4+ LOADS

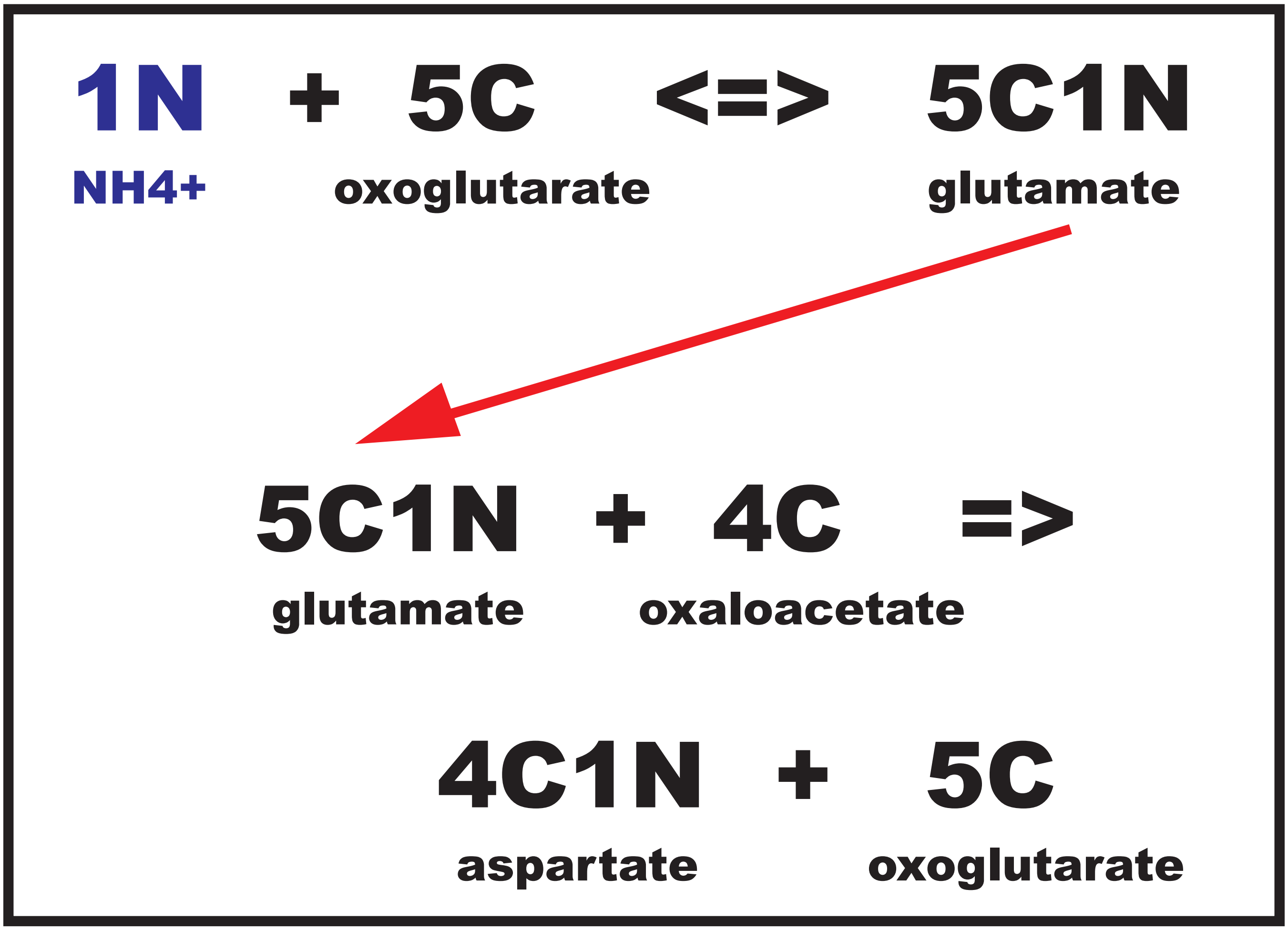

Figure 9: Ammonium assimilation under high concentration loads directly to glutamate using a carbon chain (5C), and shifting of nitrogen to aspartate using a glutamate source combined with a carbon chain (4C).

LOW ENERGY ASSIMILATION

1N	+	4C1N	=>	4C2N
NH4+		aspartate		asparagine

OR

4C1N	+	5C2N	=>
aspartate		glutamine	

4C2N	+	5C1N
asparagine		glutamate

Figure 10: Ammonium assimilation to generate asparagine, a major transport form of nitrogen, and recombination of nitrogen to generate asparagine and glutamate. Asparagine is generated under low light, low carbohydrate levels, and low energy levels.

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